EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF *GIRARDENIA ZEYLANICA* DCNE LEAF EXTRACT AGAINST CCl_4 INDUCED LIVER DAMAGE IN RATS

Dissertation submitted to

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Submitted by:

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DECLARATION

I hereby declare that this dissertation entitled **"EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF *GIRARDENIA ZEYLANICA* DCNE LEAF EXTRACT AGAINST CCl_4 INDUCED LIVER DAMAGE IN RATS"** is based on the original work carried out by me under the guidance and supervision of **Mr. G. SEKAR, M.Pharm., Asst Professor,** Department of Pharmacology for submission to the Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmacology. This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university. The information furnished in this dissertation is genuine to the best of my knowledge and belief.

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MONISHA. R.

Reg. No. 26116596

DEDICATED

TO

MY FATHER

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ANNEXURE 1

ABBREVIATIONS

SGOT	Serum glutamic pyruvic transaminase
SGPT	Serum glutamic oxaloacetic transaminase
ALP	Alkaline phosphatase
ROS	Reactive oxygen species
NASH	Non Alcoholic Steato Hepatitis
NAPQI	N-acetyl-p-benzoquinone imine
$NSAID_s$	Nonsteroidal anti-inflammatory drugs
LFTs	liver function tests
GSH	glutathione peroxidase
CAT	Catalase
GSH	glutathione peroxidase
SOD	superoxide dismutase
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
TP	Total protein
ALT	Alanine aminotransferase
AST	aspartate amino transferase
LPOx	Lipid peroxidase
IFCC	International federation of clinical chemistry and laboratory medicine
TG	Triglycerides
AST	Aspartate amino transferase

LPOx	Lipid peroxidase
LD50	Lethal Dose
OECD	Organization for economic co-operation and development
PO	Per oral
Mg/dl	Milligram per deciliter
Mg	Milligram
NADH	Nicotinamide adenine dinucleotide
BC	Before Christ
CMC	Carboxy methyl cellulose
GIT	Gastro intestinal tract
U/L	Unit per litre
ROI	Reactive oxygen intermediate
RBC	Red blood cells
GPx	Glutathione peroxidase
HbAlc	Glycosylated heamoglobin

ANNEXURE 2

Post Graduate and Research Department of Botany
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St. Thomas College, Pala
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12-12-2013

CERTIFICATE

This is to certify authentically that the specimen brought to me by Ms. Monisha R., Student, JKKMMRF College of Pharmacy, Erode, Tamil Nadu is **Girardenia zeylanica Dcne.** of the family Urticaceae. Its common name (Malayalam) is *Anachhoriayanam*

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INTRODUCTION

MEDICINAL PLANTS

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. (Biljana Bauer Petrovska, 2012) India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value.

Herbal medicine is a major component in all indigenous people's traditional medicine. In India the use of the medicinal herbs is as old as 1500BC. Medicinal herbs have been in use for thousands of years, in one form or other, under the indigenous system of medicine like Ayurveda, Unani and Sidha. India has 13,000-20,000 specious flowering plants, 2800 algae, 23,000 fungi, 1,600 types of latches, 1,800 varieties of bryophytes and an estimated 30 million types of microorganisms. There are few medicinal herbs of commercial importance, in context with the abundant flora in India. India is perhaps the large producer of medicinal herbs and is rightly called as "Botanical garden of the world". Herbal medicines are prepared from a variety of plant material such as leaves, stems, roots, barks, etc. They usually contain many biologically active ingredients and are considered to be medicinal if they possess pharmacological activities of possible therapeutic use. Today, herbal drugs are coming back into prominence and many drugs commonly used today are of

herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States of America contain at least one active ingredient derived from plant materials. Side effects of the conventional medicines such as antibiotics, antimicrobial agents are the major problem. Over the years, some of the infectious organisms have developed resistance to synthetic drugs hence more and more complications. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural product having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor people.

According to the World Health Organization (WHO) more than 1 billion people rely to some extent on medicinal plants. The WHO has listed 22,000 plants around the world having medicinal values. The extensive advances and development of science of phyto-pharmaceuticals and hopes for remedies in chronic disease generated new enthusiasm in the research workers to develop herbal medicines. The major achievement in the field was the discovery of vincristine and vinblastine in the madagascars periwinkle and it make marked difference in the treatment of cancer. Morphine, digitoxin, ergotamine and quinine are the some of the other excellent drugs that obtained from different plant species (M. Hamayan et al.2006).

LIVER

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Inspite of tremendous scientific advancement in the field of

hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. Presently, a few hepatoprotective drugs and that too from natural sources, are available for the treatment of liver disorders. Hence, people are looking at the traditional systems of medicine for remedies to hepatic disorders.(Feroz Z et al, 2013).

According to W.H.O about 25,000 people die in every year due to hepatic diseases. The common ailments of liver are cirrhosis, cholestasis, hepatitis, hepatic encephalopathy, portal hypertension, fulminate hepatic failure and certain tumors like hepatoma in liver. It is estimated that 2 billion people around the world are infected with hepatitis B virus and about 370 millions of these have the chronic form of the disease.

Medicinal herbs are significant source of pharmaceutical drug and the latest trends have shown increasing demand of phytodrugs and some medicinal herbs have been proven hepatoprotective potential. Silymarin, a flavonol lignin mixture extracted from *silybum* marianum (milk thistle) is a popular remedy for hepatic diseases. The present study is an attempt to test the hepatoprotective activity of *Girardenia zeylanica* Dcne extract.

ANATOMY OF LIVER

Liver is the largest organ in the human body with a median weight of (1600-1800 gms) in men and (1300-1400 gms) in women. The liver is a reddish brown organ and having 4 lobes of unequal shape and size. Lobes lies on the right side of the abdominal cavity beneath the diaphragm. The liver has a right and a left lobe and inside the two lobes there is a network of tubes, is called as biliary tree. Biliary tree carries bile from liver to intestine which helps the digestion. The liver lies to the right

side of the stomach and it is connected to two large blood vessels (hepatic artery and portal vein). The hepatic artery carries blood from the aorta in heart, whereas the portal vein carries blood from the gastrointestinal tract and also from the pancreas and spleen. These blood vessels are subdivide into capillaries and which then lead to a lobule. Each lobule is made up of millions of liver cells which are the basic metabolic cells and that are the basic functional units of the liver. (Lena Sibuleski, 2013)

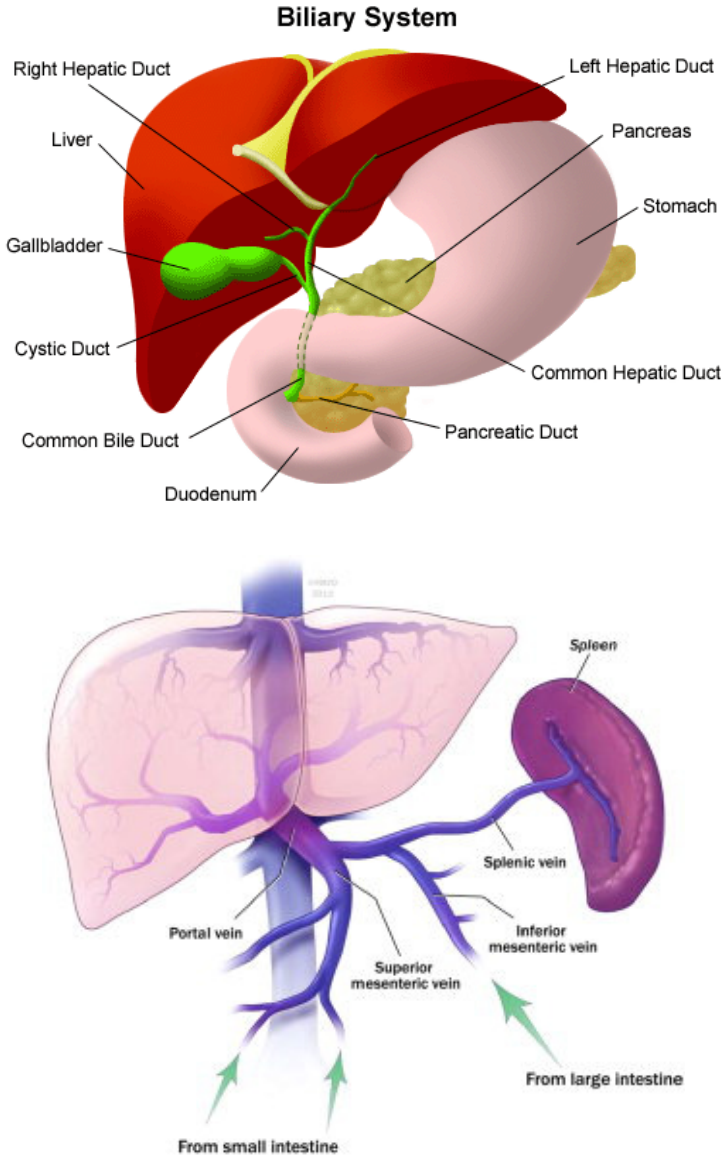


Fig. 1

Bile Ducts

The tubes that carry bile through the liver and gallbladder are known as bile ducts and form a branched structure known as the biliary tree. Bile produced by liver cells drains into microscopic canals known as bile canaliculi. The countless bile canaliculi join together into many larger bile ducts found throughout the liver.

These bile ducts next join to form the larger left and right hepatic ducts, which carry bile from the left and right lobes of the liver. Those two hepatic ducts join to form the common hepatic duct that drains all bile away from the liver. The common hepatic duct finally joins with the cystic duct from the gallbladder to form the common bile duct, carrying bile to the duodenum of the small intestine. Most of the bile produced by the liver is pushed back up the cystic duct by peristalsis to arrive in the gallbladder for storage, until it is needed for digestion.

Blood Vessels

The blood supply of the liver is unique among all organs of the body due to the hepatic portal vein system. Blood traveling to the spleen, stomach, pancreas, gallbladder, and intestine passes through capillaries in these organs and is collected into the hepatic portal vein. The hepatic portal vein then delivers this blood to the tissues of the liver where the contents of the blood are divided up into smaller vessels and processed before being passed on to the rest of the body. Blood leaving the tissues of the liver collects into the hepatic veins that lead to the vena cava and return to the heart. The liver also has its own system of arteries and arterioles that provide oxygenated blood to its tissues just like any other organ.

Lobules

The internal structure of the liver is made of around 100,000 small hexagonal functional units known as lobules. Each lobule consists of a central vein surrounded by 6 hepatic portal veins and 6 hepatic arteries. These blood vessels are connected by many capillary-like tubes called sinusoids, which extend from the portal veins and arteries to meet the central vein like spokes on a wheel.

Each sinusoid passes through liver tissue containing 2 main cell types: Kupffer cells and hepatocytes.

- *Kupffer cells* are a type of macrophage that capture and break down old, worn out red blood cells passing through the sinusoids.
- *Hepatocytes* are cuboidal epithelial cells that line the sinusoids and make up the majority of cells in the liver. Hepatocytes perform most of the liver's functions – metabolism, storage, digestion, and bile production. Tiny bile collection vessels known as bile canaliculi run parallel to the sinusoids on the other side of the hepatocytes and drain into the bile ducts of the liver.

Histology of liver

The basic functional unit of liver is the liver lobule, a cylindrical structure, several millimeters in length 0.8 mm. and 2 mm in diameter. The human liver contains 50,000 to 1, 00,000 lobules and which is constructed around a central vein which empties into a hepatic venule than the hepatic vein finally into inferior venacava. The hepatic lobule is roughly a hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the center in liver.

The hepatic acinus consists of an irregular shaped and roughly ellipsoidal mass of hepatocytes aligned around the hepatic arterioles and portal venules. The acinus is roughly divided into different zones that correspond to distance from the arterial blood supply. If hepatocytes are closest to the arterioles are the best oxygenated, while those farthest from the arterioles have poorest oxygen supply.

Hepatocytes are the main important functional cells of the liver and are polygonal in shape. Liver cells sides can be in contact either with sinusoids or neighbouring hepatocytes the mid perform a number of metabolic endocrine and secretary functions. Roughly 80 percent of the mass of the liver is contributed by hepatocytes and are exceptionally active in synthesis of protein and lipids for export and also synthesize and secrete very low density lipoproteins.

Glycogen is a polymer or glucose and the density or its aggregates in hepatocytes varies dramatically depend on whether the liver is examined shortly after the meal which shows abundant glycogen or following a prolonged fast shows minimal quantities of glycogen.

Sinusoids are the small canals formed by the plates of hepatocytes. Sinusoids are approximately 8-10 ¹m in diameter. They are orientated in a radial direction in the lobule. Sinusoids are low-pressure vascular channels that receive blood from terminal branches of the hepatic artery and portal vein at the periphery of lobules and deliver it into central veins. Sinusoids are lined with endothelial cells and flanked by plates of hepatocytes. The space between sinusoidal endothelium and hepatocytes is called the space of Disse. Sinusoidal endothelial cells are highly fenestrated, which allows virtually unimpeded flow of plasma from sinusoidal blood into the space of Disse.

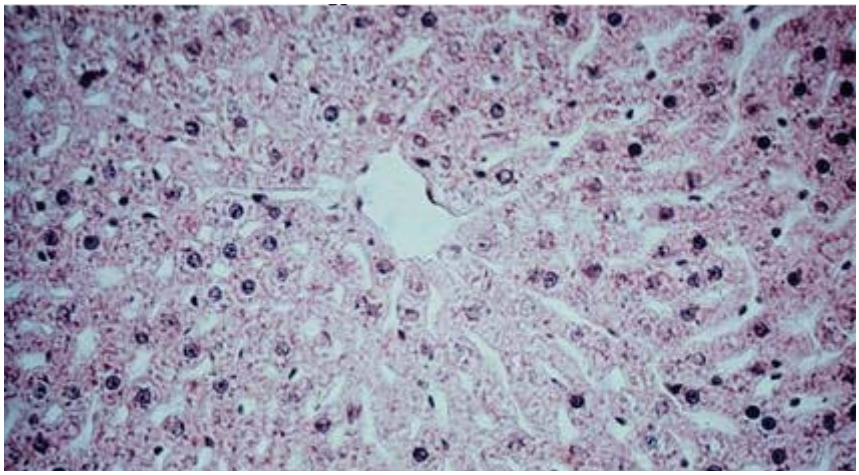


Fig. 2 Histology of liver

FUNCTIONS OF LIVER

1. Carbohydrate metabolism

- Glugconeogenesis
- Glycogen synthesis & metabolism

The liver is especially important in maintaining a normal blood glucose level in body and can break down glycogen to glucose and release glucose into the bloodstream when blood have low glucose level. The liver also can convert amino acids into lactic acid and galactose into glucose. The liver converts glucose to glycogen and triglycerides for storage when blood glucose is high, as occurs just after having a meal.

2. Lipid metabolism

- Fatty acid synthesis
- Cholesterol synthesis &excretion
- Lipoprotein synthesis.
- Ketogensis converting the fatty acid ketone bodies**.**

Hepatocytes store some triglycerides, break down fatty acids to generate ATP, synthesize lipoproteins, transport fatty acids, triglycerides and cholesterol to and from the body cells, synthesize and use cholesterol to make bile salts.

3. Protein metabolism

- Synthesis of plasma proteins.
- Urea synthesis.

Hepatocytes de-aminate (remove the amino group, NH2) amino acids so that amino acid can be used for the production ATP or converted to carbohydrate or fats. The resulting toxic ammonia is which is then converted into the much less toxic urea and excreted in urine. Hepatocytes can also synthesize most plasma proteins, such as alpha and beta globulins, albumin, prothrombin and fibrinogen.

4. Processing of drugs and hormones

- Metabolism, conjugation & excretion of steroidal & poly peptide hormones

The liver can detoxify substances such as alcohol or excrete drugs like penicillin, erythromycin, and sulfonamides into the bile. It can also chemically alter or excrete thyroid hormones and steroid hormones such as estrogen and aldosterone.

5. Excretion of Bilirubin:

Bilirubin, derived from the blood cells is absorbed by the liver from the blood stream and secreted into bile. Most of the bilirubin in bile is metabolized in the small intestine by bacteria and eliminate in to feces.

6. Phagocytosis:

The stellate reticulo-endothelial (Kupffer's) cells of the liver phagocytose aged white blood cells, red blood cells and some bacteria.

7. Storage

Liver is a good part in storage of glycogen,vitaminA,vitamin B_{12} and iron

PATHOPHYSIOLOGY OF LIVER

Liver diseases are considered as fatal & life threatening and it creates a serious challenge to public health. Liver diseases are due to infection and / or exposure of liver to various toxic substances such as different drugs or alcohol. Some times over dosage of some drugs can also lead to liver damage. Now-a-day's due to inadequacy of liver protective agents, researchers and traditional medicine practioners are concentrate in herbal based remedies for the various liver disorders. Modern medicines have little to offer for alleviation of hepatic disorders so there was no safe hepatoprotective drug available for the treatment of liver disorders. Therefore, many folk remedies from plantsource are used for the protection of hepatic disease starting from ancient period. Liver diseases remain as one of the serious health problems however we do not have satisfactory liver protective drugs in allopathicmedical practice. So herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes. Numerous medicinal plants and

their formulations are used for liver disorders in ethnomedical practices as well as traditional system of medicine in India.

Liver disease which cause an acute or chronic damage to the hepatic cells, usually caused by infection (viral), exposure to some drugs or toxic compounds, an autoimmune process, injury or by a genetic defect such as haemochromatosis. The disease can be categorized by the effect it has on the hepatic cells. Hepatitis is an inflammation of the liver mostly by virus, cirrhosis involves irreversible scarring and progressive hepatic cell death, stones can be developed and which form cancer, blockages and fatty liver, some of the cases can be life threatening. Genetic defect may cause liver disease by depositing substances in the hepatic cell or preventing vital functions of liver.

JAUNDICE

This is the yellow pigmentation of the skin, mucous membrane and deeper tissue due to increased bilirubin level in blood. The normal serum bilirubin level is 0.5 to 1.5mg%. When this exceeds 2 mg%, jaundice occurs. (Maimburg et al., 2010).

Types and Causes of Jaundice

Jaundice is classified into 3 types. Mainly Haemolytic jaundice,Hepatocellular jaundice and obstructive jaundice.

a) Haemolytic jaundice

Haemolytic jaundice is also called prehepatic jaundice. During this, the secretary function of liver is normal. But there is excessive destruction of RBCs and thus the bilirubin level of blood is increased. The liver cells excrete much bilirubin rapidly. So it accumulates in the blood resulting in jaundice.

b) Hepatocellular Jaundice

The Jaundice due to the damage of liver cell is called hepatocellular Jaundice. Here bilirubin is conjugated. But the conjugated bilirubin cannot be excreted. So it returns to the blood. The damage of liver cells occurs because of toxic substance or by infection.

c) Obstructive Jaundice

This is other wise called extra hepatic cholestatic jaundice. It is due to the obstruction of bile flow at any level of biliary system. The bile cannot be poured into small intestine and bile salts and bile pigments enter into circulation.

HEPATITIS:

- There are two major forms of hepatitis.
- In acute hepatitis the liver is damaged quickly and in chronic hepatitis the liver is damaged slowly only, over a long time.
- Hepatitis can be caused by several viruses that damage the liver, termed as hepatitis viruses. These viruses have been named according to their discovery as hepatitis **A, B, C, D, and E.**

Hepatitis A

This infection is mainly caused by consuming contaminated food or water and common victims are children. Most infected patient do not even know about they have been exposed to that particular virus.

Hepatitis B

It is fairly common disease and the virus can be spread one person to other by exposure to blood, through sexual relations, and from mother to baby. Hepatitis B still the most common cause of acute viral hepatitis. Vaccine is used prophylaxis purpose only to prevent infection.

Hepatitis C

It is less common than hepatitis B as a cause of acute hepatitis, but in some cases the majority of the people who contract it become chronically infected, hepatitis c can able to spread through contact with infected blood, Hepatitis C infection can be very serious. Most people infected experience no symptoms but the virus may remain in the liver for years and it is not discovered until much damage is done. Up to fifty percent of those infected with Hepatitis C are able to fight off the virus within six months. However, many patients become develop a chronic infection. (David Baxter, 2013)

- Hepatitis D and E are rare.

LIVER CIRRHOSIS

Anything that causes severe ongoing damage to the hepatic cells can lead to liver cirrhosis. It is marked by scar formation or cell death occur in hepatic cell and which lead to a progressive disease that creates irreversible damage of liver. It is treated by trying to limit further damage to hepatic cells. The main causes of liver cirrhosis include chronic excessive intake of alcohol, auto immune hepatitis caused by auto immune reaction, primary biliary cirrhosis persistence hepatitis C or B virus and by inherited metabolic diseases (Wilsons disease). Metabolic and clinical

abnormalities can be occur later stage only. The main causes of death in liver cirrhosis include uncontrolled bleeding spaticaemia and long prothrombin time and elevated level of bilirubin. Liver cirrhosis has no signs or symptoms in its preliminary stages, but as it progresses, it can be cause fluid build-up in the abdomen cavity called as cites, muscle wasting, bleeding from the intestines easy bruising, enlargement of the breasts in men called as gynecomastia, and a number of other problems may be occur. Liver function is monitored with such tests as albumin, bilirubin, prothrombin time and a liver panel.

GALLSTONES

In gallbladder cholesterol and bile pigments (bilirubin) in the bile may form stone. It will cause symptoms and problems which depending upon their size and location of the stone. If stone present in gallbladder for a long time, they may damage or block the duct that origins bile from the gallbladder and which causing sharp pain to develop suddenly in the upper right pan of the abdomen cavity, and in some cases, it may lead to the gallbladder fever.

OBSTRUCTION

Gallstones, trauma, tumors, and inflammation can cause blockage or obstructions in the ducts draining the liver .When an obstruction occurs, bile and its associated wastes accumulate in the liver cells and the patient's skin and eyes often turn to yellow (Jaundice) bilirubin in the urine turns to dark brown in color.

FATTY LIVER

Fatty liver which leads to enlargement of liver, tenderness, and abnormal functions of liver may be occurs. There are two major cause of fatty liver disease that

including excessive alcohol consumption and non alcoholicsteato hepatitis. In first condition of fatty liver in which common cause is intake of excessive alcohol and it is a reversible condition usually resolving with abstention from alcohol. Second cause of fatty liver is non alcoholicsteatohepatitis the most common chronic hepatitis is not caused by some viruses. It is commonly seen in overweight and diabetic patient. Excess fat in liver can be lead to inflammation, progressing to scarring (or cirrhosis) in 30 percent of patients. Treatment of fatty liver includes weight loss,regular exercise, regulating blood pressure and diabetes, cholesterol levels, limiting or stoping of alcohol consumption and not smoking.

LIVER CANCER

Liver cancer may be caused by hepatitis and cirrhosis in some limited cases only, but cancer from any other parts of the body that spreads in to the liver is more common cause. It is better patient who have chronic hepatitis disease or liver cirrhosis may be checked on a regular basis for cancer, often with an Alpha-Fetoproteins (AFP) test or an ultrasound. Infiltrative conditions which can be affect the liver include lymphomas and amyloidosis.

INHERITED ABNORMALITIES IN LIVER METABOLISM

There are four different conditions in which jaundice is caused by inherited abnormality of Bilirubin metabolism are gilberts, crigler – najjar, Dubin – Johnson& rotor syndromes. Gilbert's syndrome affects 2-3% of the population but the others are rare and the patient with Gilberts syndrome decreased conjugation of blood. The jaundice of Gilbert's syndrome is typically mild & present only intermittently. It is often noticed after an infection. The liver will be histologically normal.

- **Wilsons** disease characterized by decreased biliary excretion of copper in liver. It is an inherited abnormality of copper metabolism in liver. Copper is deposite or accumulated in the hepatic cells and patients having Wilsons diseases may present in child hood with hepatitis accompanied by renal tubular defect and haemolysis or in children with cirrhosis may be happen.

- **Hereditary Hemochromatosis –** The common adult genetic liver disease in which a specific genetic defect lead to iron accumulation in the liver cells, leading to hepatic cells cancer and liver cirrhosis in some patients. Iron accumulation may go beyond the liver affecting the joints, heart and pancreas. Specific effective treatments are available for Hemochromatosis. Liver transplantation may be required in some of these patients.

- **Alpha-1 antitrypsin deficiency (Alpha-1)** – This inherited disease affect the liver and the lungs in patient which is caused by an inability to produce enough amount of a particular protein, called Alpha-1 antitrypsin, which is used to prevent the breakdown of some enzymes in various organs. Management of Alpha-1 antitrypsin deficiency includes maintaining normal nutrition, patient education.

HEPATOTOXICITY – DIFFERENT MECHANISMS

Damage to the hepatic cells is not due to the drug itself but due to the over dosage which leads production of a toxic metabolite N-acetyl-p benzoquinonimine (NABQI or NAPQI) which is produced by cytochrome P 450 enzymes in liver cell.

In overdoses of some drugs, large amount of NAPQI is generated which overwhelm the detoxification process and leads to injury to liver cells. Nitric acid have some role in inducing liver toxicity. Hepatotoxicity caused by NSAIDs were documented to be both idiosyncratic reaction and dose dependant reaction. Eg: Intrinsic hepatotoxicity associated with aspirin and phenylbutazone and Ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin has been associated with idiosyncratic reaction. Long term use of steroid in children which lead to enlarged liver cells. (Kuester et al., 2002).

Carbon tetrachloride

Liver injury due to carbon tetrachloride in rats was reported in 1936 and has been most commonly and successfully used by many investigators. Carbon tetrachloride (CCl_4) is metabolized by cytochrome P-450 in mitochondria and endoplasmic reticulum with $CC1_3O^-$ formation, a reactive oxidative free radical, which initiates lipid peroxidation process.

$$CCl_4 \text{----------} \blacktriangleright CCl_3O^- + O^-$$

The secondary mechanisms could involve the generation of toxic products arising directly from metabolism of CCl_4 or from peroxidative degeneration of membrane lipids. The possible involvement of radical such as trichloromethyl ($\cdot CCl_3$), trichloromethylperoxy ($\cdot OOCCl_3$), and chlorine ($\cdot Cl$) free radicals, as well as

phosgene and aldehydic products of lipid peroxidation, as toxic intermediates. (Sharma Nidhi et al., 2012).

Single dose administration of $CC1_4$ to a rat produces a centrilobular necrosis and fatty changes, within 24 hours. After administration the poison reaches its maximum concentration in the liver within three hours. Thereafter, the level of CCl_4 falls and by 24 hrs there is nor remaining in the liver. The development of necrosis is associated with small leakage of hepatic enzymes into serum.

Dose of carbon tetrachloride: 0.1 to 3 ml/kg I.P.

Galactosamine

It produces diffuse type of liver damage simulating the viral hepatitis. It presumably disrupts the synthesis of essential uridylate nucleotides that cause organelle damage and cell death may happen in last stage. Reduction of those nucleotides would impede the normal synthesis of RNA and decline protein synthesis. This mechanism of toxicity brings about an increase in cell membrane permeability in hepatic cells leading to leakage of enzyme and eventually cellular death. Galactosamine may cause cholestasis and from its damaging effects on bile ducts or ductless or canalicular membrane. Galactosamine decrease the flow of bile and its content (bile salts, deoxycholic acid and cholic acid).

Dose- D-Galactosamine: 400 mg/kg, I.P.

Thioacetamide

Thioacetamide mechanism interferes with the transport of RNA from the nucleus to cytoplasm which may cause membrane damage. A thioacetamide metabolite (perhaps s-oxide) is responsible for hepatic cell damage. It will reduce rate

of oxygen consumption as well as the number of viable hepatocytes. It also decreases the amount of bile and its content (bile salts, cholic acid and deoxycholic acid).

Dose - Thioacetamide: 100 mg/kg, S.C.

Alcohol

During the oxidation process, ethanol enhances the generation of oxyfree radicals. The peroxidation of membrane lipids in liver cell results in loss of membrane integrity and structure. This results in elevated levels of membrane bound enzyme (i-glutamyltranspeptidase) in serum. Alcohol inhibits antioxidant enzyme such as glutathione peroxidase, decrease the activity of catalase, superoxide dismutase, along with increase in levels of glutathione in liver. The decrease in activity of antioxidant enzymes superoxide dismutase, glutathione peroxidise damages the liver cells. By oxidation of ethanol free radicals are produced. Alcohol pre-treatment stimulates the toxicity of carbon tetrachloride due to increased production of toxic reactive metabolites such as trichloro-methyl radical by the microsomal mixed function oxidative system. This activated free radical binds covalently to the macromolecules and induces this lipid peroxidative degradation of biomembranes is the principle reason of hepatotoxity.

Paracetamol

Paracetamol, a commonly used analgesic and antipyretic agent, over dosage which will damage the liver is not due to the drug itself but a toxic metabolite, example (N-acetyl-p-benzoquinone imine NAPQI, or NABQI) which is produced by cytochrome P-450 enzymes in the liver. In normal dose, the drug is detoxified by conjugating with glutathione in phase 2 reaction but in an overdose, a large amount of

N-acetyl-p-benzoquinoneimine is generated which overwhelms the detoxification process and leads to centrilobular hepatocytes necrosis which is characterized by nuclear eosinophilic and pyknosis cytoplasm followed by hepatic lesion. The covalent binding of paracetamol toxic metabolite, N- acetyl-P-benzoquinonimine to sulphydrylgroups of the protein, result in lipid peroxidative degradation of glutathione (antioxidant enzyme) level that also leads to cell necrosis in the liver cells.

Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs have emerged as a major group of drugs that exhibiting both dose-dependent and idiosyncratic reactions which leads to chronic liver damage. Example-idiosyncratic reaction has been related to ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin and aspirin and phenylbutazone are related to intrinsic hepatotoxicity. (Aashih Pandit et al., 2012).

Glucocorticoids

Glucocorticoids affect on the carbohydrate mechanism. Glucocorticoids promote glycogen storage in the liver. Long-term steroid use in children leads to the enlarged liver which is a rare side effect. Steatosis may be happen in prolonged usage both in adult and paediatric population.

Isoniazid

Isoniazide (INH) is one of the most commonly used in combination therapy for tuberculosis. It is associated with mild elevation of liver enzymes in up to 20 percentage of patients and severe hepatotoxicity in 1-20 percentage of patients.

Natural products

Example for natural products include many amanita mushroom and aflatoxins

LIVER FUNCTION TESTS

As the liver performs it's different functions and it makes chemicals that pass into the bloodstream and bile. There are large variety of liver disorders which can alter the blood level of these chemicals. Some of these chemicals can be measured in the blood called liver function tests (LFTs).

SERUM BILIRUBIN

Bilirubin derived from haemoglobin degradation process from the red blood cells (RBC).It is an endogenous anion. Measurement of bilirubin level in blood by direct and indirect bilirubin method. It is based on the original Van der Bergh method. Bilirubin values will alter when exposed to light. So serum and plasma samples must be kept in dark before measurements. When the liver function tests are abnormal (serum bilirubin levels more than17µmol/L) it suggest that liver having some disease. (Friedman SF et.al. 2003)

Types of bilirubin

i. Total bilirubin: Normal range is 0.2-0.9 mg/dl (2-15µmol/L). This is measured as the amount, which will reacts in 30 minutes after addition of alcohol and it is higher slightly (3-4 µmol/L) in males as compared to females. This helps to diagnose Gilbert syndrome in males easily.

ii. Direct Bilirubin: Normal range 0.3mg/dl (5.1µmol/L). This is the water-soluble fraction. Direct bilirubin is measured by the reaction with diazotized sulfanilic acid in 1 minute and it gives estimation of conjugated bilirubin.

iii. Indirect bilirubin: This fraction is calculated by the difference of the direct bilirubin and total bilirubin and it is a measure of unconjugated fraction of bilirubin. (Daniel SP, Marshall MK.1999)

Any elevation in bilirubin level which shows the depletion of hepatic clearance. Mild elevation is due to inherited hyperbilirubinemias, physiological jaundice and moderate elevation due to some drugs, inherited hyperbilirubinemias, and viral hepatitis. In diazo method, bilirubin estimation is not very accurate especially when used in detecting low levels of bilirubin. Direct bilirubin method over estimates bilirubin esters at low bilirubin levels and under estimates them at high concentration.(Shivaraj Gawda et.al., 2009)

ENZYMES THAT DETECT HEPATOCELLULAR NECROSIS

Aminotransferases

The aminotransferases (formerly transaminases) are specific indicators of hepatocellular necrosis. These enzymes- aspartateamino transferase (AST, formerly serum glutamateoxaloacetic transaminase-S GOT) and alanine aminotransferase (ALT, formerly serum glutamic pyruvatetransaminase-SGPT) catalyze the transfer of the aminoacids of aspartate and alanine respectively to the á ketogroup of ketoglutaric acid. ALT is primarily localized to the liver but the AST is present in a wide variety of tissues like the heart, kidney, skeletal muscle, brain and liver. (B.R. Thapa, Anuj Walia, 2007).

Alanine transaminase (ALT)

It is an enzyme that helps to speed up chemical reactions in liver. Large amounts of ALT occur in liver cells but the blood level of ALT usually rises when the liver is injured or inflamed (such as in hepatitis), and elevated level in ALT which shows leakage from damaged tissue, and the marked elevation may be caused by hepatitis, autoimmune disease, toxic, neonatal hepatitis and ischemia.

Aspartate aminotransferase (AST)

This is an enzyme usually found inside liver cells. However if heart or skeletal muscle is damaged AST can also be released. If the patient blood test result showing high levels of aspartate aminotransferase usually means the liver is damaged in some way. For this reason ALT is usually considered to be more specifically related to liver problems.

Alkaline phosphatase (ALP)

The blood level alkaline phosphatases is raised in some types of liver and bone disease. Alkaline phosphatases are zinc metalo-enzymes and in liver, it is found in microvilli of bile canaliculi and also in the sinusoidal surface of hepatocyte.

Average level of alkaline phosphatase may be varying with age. Example lower level in middle age and relatively high in childhood and puberty and higher again in old age. Higher values alkaline phosphatase in males when compared to females. The levels of the enzyme correlate inversely with the height of the person, and directly with person's weight (Kuldp Singh, 2013). The mechanism by which alkaline phosphatase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions, (M. Adak,

Shivapuri, 2010), and the other hypothesis in acute viral hepatitis alkaline phosphatise level is usually either normal or moderately increased because of damaged liver fails to excrete alkaline phosphatase made in bone, intestine and liver. Tumours may secrete alkaline phosphatase into plasma and there are tumour specific isoenzymes such as Regan, Nagao and Kasahara isoenzymes. In patients with cirrhosis elevated serum levels of intestinal alkaline phosphatase have been found, particularly those with blood group type O, and may be associated specifically with intra hepatic disease (M. Adak, Shivapuri 2010).

Elevated levels of alkaline phosphatase also may present in patient with hepatic and bony metastasis. However some hepatic diseases may also cause a rise in alkaline phosphatase level. Example are infiltrative liver diseases, abscesses, granulomatous liver disease and amyloidosis. In cirrhosis and hepatitis of congestive cardiac failure mildly elevated levels of alkaline phosphatase may seen. In patient with hypothyroidism, pernicious anemia, zinc deficiency and congenital hypophosphatasia levels of alkaline phosphatase will be low.

Albumin

This is the major protein in blood which can bind most of the drugs which will resulting increasing the duration of action of that specific drug. Albumin synthesis in liver and it release in to blood stream. Some of the liver disorder may affect the ability to make albumin (and other proteins). Albumin synthesis stimulated by corticosteroids and thyroid hormone. In some liver disorders like cirrhosis, ascites a low level of blood albumin occurs.

Total protein

This measures albumin and all other proteins in blood including alpha 1 antitrypsin, alpha feto protein.

Bilirubin

This chemical gives bile its yellow/green colour. A high level of bilirubin in blood will make jaundiced ('yellow'). Bilirubin is made from haemoglobin. Haemoglobin is a chemical in red blood cells that is released when the red blood cells break down. Liver cells take in bilirubin and attach sugar molecules to it. This is then called 'conjugated' bilirub in which is passed into the bile ducts.

A raised blood level of 'conjugated' bilirubin occurs in various liver and bile duct conditions. It is particularly high if the flow of bile is blocked. For example, by a gallstone stuck in the common bile duct, or by a tumour in the pancreas. It can also be raised with hepatitis, liver injury, or long-term alcohol abuse.

A raised level of 'unconjugated' bilirubin occurs when there is excessive breakdown of red blood cells. For example, in haemolytic anaemia.

Role of Liver function tests

- To diagnose liver disorders.
- To monitor the activity and severity of liver disorders.
- As a routine precaution after starting certain medicines to check that they are not causing liver damage as a side-effect.

FREE RADICALS

Types of free radicals

Free radicals can be defined as molecules containing a single unpaired electron in atomic or molecular orbits. These molecules are highly reactive ion and have an important role in the pathogenesis of tissue damage in various disorders such as hepatic dysfunction, mastitis, kidney damage, inflammation, immune injury and carcinogenesis (AbdEllah, 2010). The most important free radicals include superoxide anion (O2.−), hydroxyl radical (.OH), and hypochlorous acid (HOCL) (Stohs, 1995). HOCL is produced by the reaction of hydrogenperoxide (H_2O_2) with chloride ions and plays an important role in the leukocyte respiratoryburst, which is involved in the host defense system (Lunec, 1990). Nitric oxide (NO**.**) acts as a free radical and as a biological mediator in biochemical reactions. Physiologically it is synthesized from L-arginine by NO synthase employing cofactor NADPH. In the host, NO arises in some pathological situations, such as sepsis, stroke, myocardial depression, and inflammatory responses. (Bredt & Snyder,1994)

Superoxide anion induces important reducing reactions in biological materials via Fenton like reactions, which are catalyzed by redox cycling metal ions, including iron, copper, chromium and vanadium (Stohs & Bagchi, 1995). These metal ions have the ability to accept and donate single electrons, making them important catalysts of free radical reactions, the most widely distributed and most commonly studied transition metal ions are the cations iron and copper, (Stohs,1995). Superoxide anion reduces Fe3+ in metalloproteins such as ferritin. The reduction of protein bound iron is an important reaction in biological material, because if there is sufficient H_2O_2 available, a reaction between the resultant Fe2+ and H_2O_2 occurs and gives rise to

the highly reactive **.**OH (Lunec, 1990). H_2O_2 traverses biological membranes and intracellularly targets phospholipids, carbohydrates, metalloproteins and DNA, and causes damage via Fenton's reaction. The main characteristics are short living, unstable, react with other molecules to achieve stability.

Sources of free radicals

Free radicals may be released due to the by-product of cellular respiration. synthesized by enzyme system (phagocytic cell, neutrophils and macrophages), exposure to ionizing radiation, smoking, herbicides, pesticides, fried foods or the production by chain reaction and normal cellular respiration,

Free radicals may be produced in the liver as a subsequence to hepatic detoxification of drugs, chemicals and toxic materials, (Feheret al.1992) (Ogino & Okada, 1995). The formation of oxygen free radicals may be physiological as in phagocytosis (superoxide and H_2O_2 are used by phagocytic cells to kill bacteria), a side effect of metabolic pathways, or may occur in pathological conditions due to toxic agents as in the case of ischemia, inflammation, disease, or due to decreased antioxidant defences.

Mitochondria considered a major source for the production of O_2**.-** and H_2O_2, about 2-3% of consumed oxygen is constantly converted into reactive oxygen/reactive nitrogen species (ROS/RNS) in the mitochondria, hepatocytes contain many mitochondria and therefore, generate excess ROS/RNS (Stohs,1995).

In many liver diseases, including the wide range of neonatal hepatitis, the tissue inflammatory infiltrates are likely to be responsible for the formation of O_2.-, H_2O_2, OH, HOCl and the highly cytotoxic monochloramine.

In turn, the superoxide anion attracts further neutrophils to the inflammatory site by a chemotactic activity, causing an increase in tissue injury. In addition, activated macrophages, Kupffer cells and vascular endothelium can generate nitric oxide, which may react with superoxide generating peroxynitrite. The latter is responsible for the inhibition of mitochondrial respiration and DNA synthesis.

Liver damage due to iron (hemochromatosis) and copper overload is believed, at least partially, to derive from the catalytic activity of these metals in the Fenton reaction leading to the generation of ROS and increased lipid peroxidation with consequent abnormal mitochondrial function.

Excessive free radical production has been involved in the occurrence in several disease processes like drug toxicities, inflammation, aging etc.

Antioxidants

The cells contain a variety of antioxidant mechanisms that play a central role in the protection against reactive oxygen species (Halliwell et al. 1991). The antioxidant system consists of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)), glutathione, ancillary enzymes (glutathionereductase (GR), glutathione S-transferase, and glucose 6-phosphate dehydrogenase (G6PD)),metal-binding proteins (transferrin, ceruloplasmin and albumin), vitamins (α-tocopherol, ascorbate and beta-carotene), flavonoids, and urate (Halliwell, 1994). Pathological free radical reactions do not necessarily cause cell and

tissue damage, as antioxidants of cells and tissues are able to prevent free radical injury. (Feher et al,1992)

Hepatic oxidative stress and lipid peroxidation

The term "oxidative stress" has been coined to represent a shift towards the pro-oxidants in the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism. Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation, or strenuous physical activity. Protection against all of these processes is dependent upon the adequacy of various antioxidant substances that are derived either directly or indirectly from the diet.

Consequently, an inadequate intake of antioxidant nutrients may compromise antioxidant potential, thus compounding overall oxidative stress.

Oxidative stress results when reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms (Sies, 1991) and/or from a decrease in antioxidant defense, which may lead to damage of biological macromolecules and disruption of normal metabolism and physiology. (Trevisan et al, 2001). This condition can contribute and/or lead to the onset of health disorders and play a damaging role in a number of liver disorders, for example, in anoxic and reoxygenation injury during transplantation, activated phagocytes and xanthine oxidase formed during ischemia, catalyze the formation of superoxide during reperfusion.

Lipid peroxidation is implicated in the pathogenesis of several hepatic disorders in human and animals (AbdEllah et al., 2004). Hepatic failure in cattle was associated with decreased antioxidant mechanisms inside the cells, which led to the increase in the reactive oxygen species, especially H_2O_2. The decrease in hepatic

GSH-Px activity in severe fatty degeneration, for example, results in the increase H_2O_2 (AbdEllah et al., 2004), which can initiate free radical formation through Fenton's reaction. In addition, the decrease in hepatic vitamin E level, which is an important chain breaking antioxidant, results in lipid peroxidation and failure to regenerate the ascorbic acid (Mudroň et al., 1997, 1999). Increased hepatic oxidative stress was also reported in cows suffering from glycogen degeneration (AbdEllah et al., 2004), sawdust liver and liver abscesses (Sayed et al., 2003). The authors contended that the antioxidant defense was high in the case of sawdust liver, glycogen degeneration, and liver abscess, which indicated that the body can combat the increased free radical stress.

Liver abscesses in fattening steers occur mainly due to intensive feeding of highly concentrated rations. Consumption of a carbohydrate-rich diet stimulates G6PD expression in endothelial and parenchymal cells (Spolarics, 1999). Since G6PD supports reactive oxygen metabolism, the response may represent an antioxidant pathway in the hepatic cell populations that targets sinusoid born reactive oxygen species during infections (Spolarics, 1999; AbdEllah et al., 2002).

ANTIOXIDANTS AND IT'S ROLE IN LIVER PROTECTION

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases (Langseth, L, 1993). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves.

Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur with in seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being.

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage.

ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.
- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.
- Xenobiotic metabolism, i.e., detoxification of toxic substances.

Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of “leaky gut” syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body’s oxidant load.

ANTIOXIDANT PROTECTION

To protect the liver cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

These components include:

- Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids and other low molecular weight compounds such as glutathione and lipoic acid.
- Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
- Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
- Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

DIETARY ANTIOXIDANTS

Vitamin C, vitamin E, beta carotene and lycopene are among the most widely studied dietary antioxidants. Lycopene is carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables. Water-soluble antioxidant eg- vitamin C is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Lipid-soluble antioxidant vitamin E, a major, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation.

ENDOGENOUS ANTIOXIDANTS

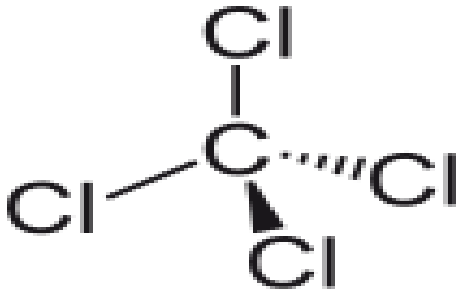
In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against freeradical-induced cell damage. The antioxidant enzymes – glutathione peroxidase, catalase and superoxide dismutase (SOD) – metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms (Duthie, G.G., and Brown, K.M., 1994) Research indicates that consumption and absorption of these important trace minerals may decrease with aging. Intensive agricultural methods have also resulted in significant depletion of these valuable trace minerals in our soils and the foods grown in them. Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism.

Free radicals are highly reactive substances produced continuously during metabolic processes. They participate mainly in physiological events such as the immune response, metabolism of unsaturated fatty acids and inflammatory reaction. The balance between free radicals and antioxidants is disrupted in many diseases. This disruption may be attributed to a number of factors such as the inability of the cells to produce sufficient amounts of antioxidants, the nutritional deficiency of minerals or vitamins, and the excess production of reactive oxygen species. (AbdEllah, 2010).

DRUG PROFILE

CARBON TETRACHLORIDE

Chemical Structure



Molecular formula	:	CCl_4
Molecular mass	:	158.82 gm/mol
IUPAC Name	:	Tetrachloromethane
Other names	:	Benziform, Carbon Tet, Tetrazol
Appearance	:	Colourless liquid
Odor	:	Ether like odor.
Density	:	1.5867 gm/cm^3
Melting point	:	-22.92º C
Boiling point	:	76.72º C
Solubility	:	Soluble in alcohol, ether, $CHCl_3$, Benzene.

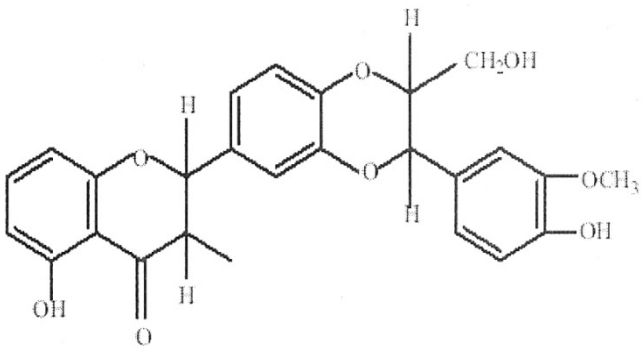
USES

It was widely used as a dry refrigerant, cleaning solvent, in detection of Neutrinos. It is one of the most potent hepatotoxins and is widely used in scientific research to evaluate hepatoprotective agents.

SILYMARIN (reference drug)

A mixture of the isomers Silibinin, Silicrystin and Silidianin and the active principle from the fruit of *Silybum marianum (Carduus marianus)* (Compositae).The principle components are the Silibinin, silicrystin, silidianin and flavonolignans of which Silibinin is the major component.

Chemical Structure of Silymarin



PICTURE OF SILYMARIN

Fig. 3

Chemical Name	:	2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl) – 2(hydroxymethyl)-6-(3,5,7-trihydroxy-4- oxobenzopyran-2-yl) benzodioxin.
Molecular Formula	:	$C_{25}H_{22}O_{10}$
Molecular Weight	:	482.44
Scientific name	:	Milk Thistle
Other Names	:	Carduimariae, Mary Thistle, Holy Thistle, Lady's Thistle, Marian Thistle, Mariendistel, Our Lady's Thistle, Silimarina, Carduusmarianum, Silybin, Silybummarianum, St. Mary Thistle, Legalon

PROPERTIES

Hepato-protective: Protects liver cells from incoming toxins, thereby also allowing it to more effectively process and release toxins that are already built up in system.

Hepato-tonic: Strengthen the liver to function more effectively.

Anti-depressant: By helping to move stagnant liver energy.

Demulcent: Soothes and moistens bladder and kidneys irritations, as well as mucous membranes and inflammations of the skin and integumentary system.

Laxative: Mildly lubricates the bowels.

HEPATOPROTECTIVE ROLE OF SILYMARIN

Silymarin has shown differing degrees of effectiveness for protecting the liver from damage caused by some drugs, alcohol, chemicals, diseases and poisonous plants. It is used to treat both acute conditions (such as poisoning) and long term diseases (such as Hepatitis C). (**Pradhan & Girish, 2006**)

- Silymarin and other chemicals in milk thistle are believed to protect liver cells in several different ways.
- Silymarin has antioxidant properties/free radical scavenging effect and has been used for the treatment of liver disorders.
- Anti-inflammatory effects of Silymarin help in preventing hepatic cells from swelling after being injured.
- Silymarin seems to encourage the liver to grow new cells, while discouraging the formation of inactive fibrous tissue.
- By changing the outside layer of liver cells, Silymarin actually may keep away certain harmful chemicals from getting into liver cells.
- Milk thistle may also cause the immune system to be more active

LITERATURE REVIEW

1) **P.S. Bedi et al.,** Reported that invitro antimicrobial activity of solvent extracts of *Girardenia diversifolia* solvent was studied and reported to have antimicrobial activity against bacterial infection caused by E.coli and against fungal disease, caused by Aspegillus Niger.

2) **S.B. Mada et al.,** have studied the hepatoprotective activity of *Mormodica charntia* leaves extract against carbon tetrachloride induced hepatotoxicity in rats and reported that the hepatoprotective activity of extract of leaves may be attributed to the antioxidant property of the plant.

3) **M. Pushpalatha and T. Ananthi,** reported that the ethanolic extract of *Solanum pubescens* showed hepatoprotective and antioxidant potential against carbon tetrachloride induced hepatotoxicity in Albino rats by judging the serum marker enzymes and antioxidant levels in liver tissues.

4) **HaiZhongHuo et al.,** have studied the hepatoprotective and antioxidant effects of Licorice extract and reported that it has showed remarkable hepatoprotective and antioxidant effect against carbon tetrachloride induced oxidative damage in Rats.

5) **NishaTripathi et al.,** reported that the phytochemical studies from the roots of *Girardinia heterohylla* proved the presence of γ - sitosterol in its roots. The γ - sitosterol is reported to have antihyperglycemic effect in STZ – induced diabetes in Rats.

RESEARCH ENVISAGED

AIM OF PRESENT STUDY

In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications. Looking to the scope of herbal drugs and increasing its demand especially in disease of liver, hypertension, diabetes, cancer, arthritis and skin disease etc, it was planned to study *Girardinia zeylanica* Dcne in detail due its effectiveness for liver problems, especially for jaundice.

Girardinia zeylanica Dcne, selected for study was made on the basis of its,

- Folklore claiming its therapeutic use as liver protection and for jaundice.
- Degree of research work which is not done.

Very less pharmacological studies have been carried out on the *Girardinia zeylanica* Dcne. Hence, we have decided to submit the project on *Girardinia zeylanica* Dcne which includes detailed Pharmacognostical, Preliminary Phytochemical and Pharmacological (Acute oral toxicity study, Hepatoprotective activity with antioxidant effect) studies to provide scientific validation to its folklore claims.

PLAN OF WORK

The plan of work for the study of *Girardinia zeylanica* Dcne was carried out as follow.

1. Collection and authentication of plant material.

2. Pharmacognostical studies

   a) Ash value
   b) Extractive value
   c) Loss on drying

3. Preliminary Phytochemical studies

   a) Preparation of extract
   b) Qualitative phytochemical studies

4. Pharmacological studies

   a) Screening of Hepatoprotective activity,

   - Assessment of Liver Function Tests.
   - Assessment of Antioxidant parameters.
   - Histopathology of liver.

COLLECTION AND IDENTIFICATION

COLLECTION OF SPECIMEN

The species for the proposed study that is leaves of *Girardinia zeylanica* Dcne were collected carefully from National park of Mathikettan, Idukki district, Kerala.

TAXONOMICAL IDENTIFICATION:

The plant was collected from National park of Mathikettan, Idukki district of kerala. The plant was positively identified by Dr. JomyAugustine, M.sc, M.Ed., PhD, Professor and Head of the Department of Botany St.Thomas College, Pala, Kottayam and authenticated the plant as *Girardenia zeylanica* Dcne of family Urticaceae from available literature.

TREATMENT OF PLANT MATERIAL:

The leaves of *Girardenia zeylanica* Dcne were washed thoroughly with water to remove the mud or dust, then it was shade dried completely. The dried leaves of *Girardenia zeylanica* Dcne was then powdered by means of mixer grinder and was sieved through sieve no.60 to get the coarse powder, which was used for further detailed studies, extraction with solvent and phytochemical studies.

BOTANICAL INFORMATION

GENERAL INFORMATION:

Common name - Nilgiri Nettle, Himalayan Giant Nettle

Binomial name - *Girardenia zeylanica* Dcne

Family - Urticaceae

TAXONOMICAL CLASSIFICATION

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliatae

Order - Urticales

Family - Urticaceae

Genus - Girardinia

Species - *G. zeylanica*

VERNACULAR NAMES:

Common name - Himalayan Giant Nettle, Nilgiri Nettle

Malayalam - Anachoriyanam, Kuttithoova

Tamil - Chenthotti

Hindi - Bichua, Alla, Awa

Geographical source:

Girardenia zeylanica Dcne is found abundantly in open forest land and river side of moist habitat in Himalayan parts of India like Uttarakhand, Himachal Pradesh and J&K and in high altitudes of Western Ghats and in Nepal. It grows naturally within elevations of 1,200 to 3,000 metres (3,900 to 9,800 feet). It is a shade bearing, tall, stout and erect herb growing up to 3m height with perennial roots stock. The plant is found on clump and each clump has many stem. The stem bark contains fibers of unique quality which is strong, smooth and light.

Description:

A tall robust, perennial-rooted herb, 1.2-1.3 m. high, closely armed with slender stinging hairs, stem and branches furrowed. Leaves alternate, 3-nerved, entire or lobed, serrate; stipules connate, foliaceous. Flowers monoecious, in simple or panicled cymes or heads armed with stinging hairs. Perianth simple.

Leaves 10-25 cm. long and often as broad, the upper variously lobed and more or less deeply cut though never to the base, more or less - pubescent, coarsely serrate, the serratures triangular, sometimes 13 mm. deep, petioles 7.5-18 cm. long, armed with stinging hairs; stipules 13-16 by 8 mm., ovate, cordate, shortly acuminate, 2-fid. Flowfers in pedunculateracemose cymes, the males in the lower, the females in the upper axils. (C.P. Khare, 2007).

Photographs of *Girardenia zeylanica* Dcne

Fig. 4

Fig. 5 – Stinging hairs of *G. zeylanica* leaves

Chemical constituents:

Girardenia zeylanica Dcne contain alkaloids, glycosides, flavonoids and steroids. An analysis of leaves and stems revealed the following: moisture 72.5%, protein 1.4%, fat 0.3%, carbohydrate 2.6%, fibers 20.9%, ash 1.8%, calcium 0.2%, phosphorus 0.06%. Leaves are rich in vitamin C and A, and also contain vitamin B.

The ash is rich in sulfate and potassium chloride. Stigmasta-4,22-dien-3-one, stigmasterol, stigmasteryl stearate, and palmitic acids are reported in *Girardinia zeylanica* Dcne Plant gave 2-di-C-glcosylflavones of vicenin and lucenin type, anthocyanin-cynidin-3-glucoside, luteolin-7-glycoside and mono-C-glcosyl flavones – vitexin and orientin.

Medicinal uses:

The plant is considered antitubercular, antiseptic and antidysentric. In Rwanda *Girardenia zeylanica* Dcne is part of an antidote against snakebites, and in veterinary medicine a maceration of the leaves is used for treatment of theileriasis. In Kenya the root is boiled in goat's bone soup, which is drunk to gain strength. In Nepal leaf preparations are used for the treatment of headache, fever and swollen joints, and juice of the root is given in case of constipation. Ash of the plant is applied externally for the treatment of ringworm and eczema. In north-eastern India the seeds are used as fishpoison.

MATERIALS AND METHODS

PHARMACOGNOSTICAL STUDIES:

Ash values, extractive value, loss on drying are used for the study of physical properties.

ANALYTICAL PARAMETERS

➢ ASH VALUES

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form.

The ash content of a crude drug is generally taken to be the residue remaining after incineration. Ash standards have been established for a number of official drugs. Usually these standards get a maximum limit on the total ash or on the acid insoluble ash permitted.

The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.

The ash or residue yielded by an organic chemical compound is as a rule, a measure of the amount of inorganic matters present as impurity. In most cases, the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drugs in powder form.

Procedures given in Indian pharmacopoeia were used to determine the different ash values such as total ash and acid insoluble ash.

Determination of total ash value:

Weighed accurately about 3 gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon. Cooled and weighted and then calculated the percentage of total ash with reference to the air dried drug.

Determination of acid insoluble ash value:

The ash obtained as directed under total ash above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on ashless filter paper, washed with hot water ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of Water soluble ash value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450˚C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

Determination of sulphated ash value:

About 3 gms of accurately weighed air dried powdered drug was taken in a tared silica crucible which was previously ignited and weighed. Then ignite gently at first until the drug was thoroughly charred. The crucible was cooled and residue was moistened with 1 ml of concentrated sulphuric acid, heated gently until the white

fumes were no longer evolved and ignited at 800 ± 25º C until the black particles has disappeared. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed cooling and weighed to get a constant weight. The percentage of sulphated ash was calculated with reference to the air dried drug. All the ash values were calculated and recorded.

➢ EXTRACTIVE VALUES

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extractive value

5 gm of the air-dried coarse powder of *Girardenia zeylanica* Dcne was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air- dried drug. The results are recorded in the table.

Determination of water soluble extractive value

Weigh accurately 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to

dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug. The results are given in the table.

- **LOSS ON DRYING**

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Desiccators or hot air oven). If the sample is in the form of large crystals, then reduce the size by quick crushing to a powder.

Procedure

About 1.5 gm of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105˚C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of leaf of *Girardenia zeylanica* Dcne **:**

About 250 gm of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days to remove fatty substances. At the end of 2nd day the powder was taken out and it was dried. After drying it was again packed and extracted by using ethanol as solvent, till colour disappeared. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness.

S.No.	Name of extract	Colour	Consistency	Yield% W/W
1	Ethanolic extract	Dark greenish	Sticky mass	12.5

Table: 1. Nature and colour of ethanol extract of *Girardenia zeylanica* Dcne**.**

CHEMICAL TESTS:

A) Test for carbohydrates

1. **Molisch Test:** It consists of treating the compounds with -naphthol and concentrated sulphuric acid along the sides of the test tube.

   Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C.K *et al*, 2000)

2. **Fehling's Test:** Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

3. **Benedict's test:** To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.

4. **Barfoed's test:** To the 5ml of the Barfoed's solution add 0.5ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

B) Test for Alkaloids

1. **Dragendroff's Test:** To the extract, add 1ml of Dragendroff's reagent. Orange red precipitate is produced.

2. **Wagner's test:** To the extract add Wagner reagent. Reddish brown precipitate is produced.

3. **Mayer's Test:** To the extract add 1ml or 2ml of Mayer's reagent. Dull white precipitate is produced.

4. **Hager's Test:** To the extract add 3ml of Hager's reagent yellow Precipitate is produced.

C) Test for Steroids and Sterols

1. **Liebermann Burchard test:** Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. **Salkowski test:** Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides

1. **Legal's test:** Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.

2. **Baljet test:** To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.

3. **Borntrager test:** Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer

is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. **Killer Killani test:** Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids

Shinoda test: Red colour is produced when the sample, magnesium turnings and then concentrated hydrochloric acid is added.

G) Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H)Tests for Tannins and Phenolic Compounds:

The Phenol content in the raw material of *Girardenia zeylanica* Dcne was estimated by spectroscopically.

To 2-3 ml of extract, add few drops of following reagents:

a) 5% $FeCl_3$ solution: deep blue-black color.

b) Lead acetate solution: white precipitate.

c) Gelatin solution: white precipitate

d) Bromine water: decolouration of bromine water.

e) Acetic acid solution: red color solution

f) Dilute iodine solution: transient red color.

g) Dilute HNO_3: reddish to yellow color.

I) Test for Fixed Oils and Fatty acids

a) Spot test:

Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

b) Saponification test:

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours.Soap formation indicates the presence of fixed oils and fats in the extracts.

J) Test for Gums and Mucilage:

a) Ruthenium red test:

Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage.

K) Test for Proteins and Amino acids

Biuret test: Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

Ninhydrin test: Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

TOXICOLOGICAL EVALUATION

Determination of LD_{50} value

Acute Oral Toxicity Study

The procedure was followed by using OECD guidelines 423 (Acute toxic class method).

The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the OECD for the classification of chemical which cause acute toxicity.

Procedure:

Twelve animals (Wister Albino rats, 150-200gm) were selected for studies.The starting dose level of *Girardenia zeylanica* Dcne extract was 100 mg/kg body weight per oral.Most of the crude extracts possess LD_{50} value more than 2000 mg. /kg of the body weight of animal used.

Dose volume was administered 0.1 ml / 100 gm. body weight to the animal by oral route. After giving the dose the toxic signs were observed within 3-4 hours.

Body weight of animals before and after administration, onset of toxicity and signs of toxicity like changes in skin and fur, eyes, and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somato-motor activity and behavior pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted, if any, was observed.

PHARMACOLOGICAL EVALUATION

Animals:

Healthy male Wistar albino rats of 2 to 3 months of age and approximately weighing between 150-250g were used in the present study. Rats were housed in polypropylene cages and allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature 25±2ºC.

All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The experimental protocol has been approved by institutional animal ethics committee, JKKMMRF College of Pharmacy, B.Komarapalayam, Namakkal. (Regd. No. JKKMMRFCP/1158/PO/ac/07CPCSEA)

EXPERIMENTAL PROTOCOL:

The rats were divided into five groups, comprising of six animals in each group.

Group I : Received 5% CMC 10ml/kg body weight. The group served as a normal control.

Group II : Received CCl_4 (0.7 ml/kg) body weight intraperitonially.

Group III : Received Silymarin, the standard drug (25mg/kg).

Group IV : Received ethanolic extract of leaves of *Girardenia zeylanica* 100mg/Kg.

Group V : Received ethanolic extract of leaves of *Girardenia zeylanica* 200mg/Kg.

0.7 ml/kg of CCl_4 was injected intra peritoneally (i.p.) to all groups except normal control to induce hepatotoxicity on 3, 6 and 10thdays of experiment. Duration of the study was 10 days. All the animals were sacrificed on the 11th day for the estimation of biochemical parameters.

Estimation of serum biochemical parameters:

On 11^{th} day blood was collected from animals under anaesthesia by cardiac puncture. Blood samples collected was centrifuged at 3500 rpm for 15 mins at room temperature for separation of serum. The clear, non-haemolysed sera was separated using clean dry disposable plastic syringe and stored at -20ºC for measurements of the following,

Liver function test:

- Serum glutamic pyruvic transaminase (SGPT)
- Serum glutamic oxaloacetic transaminase (SGOT)
- Alkaline phosphatase (ALP)
- Total protein (TP)
- Total bilirubin (TB)

Lipid profiles:

- Cholesterol
- Triglycerides (TG)
- High Density Lipoprotein (HDL)
- Low Density Lipoprotein (LDL) and
- Very Low Density Lipoprotein (VLDL)

EVALUATION OF SERUM PARAMETERS:

All the above biochemical parameters were estimated using serum. All the above biochemical parameters were estimated using semi-auto analyzer (Photometer 5010 $_{v5+}$, Germany) with enzymatic kits procured from Primal Healthcare limited, Lab Diagnostic Division, Mumbai, India.

A) ESTIMATION OF SGPT

Serum Glutamate Pyruvate Transaminase (SGPT)

Principle

L-Alanine +2-oxoglutarate $\xleftrightarrow{\text{ALAT}}$ L-Glutamate + Pyruvate

Pyruvate + NADH + H^+ $\longleftrightarrow$ D-Lactate + NAD^+

Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, eg.from patients with myocardial infarction, liver diseases and intensive care patients.

Method

Kinetic UV test, according to the international federation of clinical chemistry and laboratory medicine (IFCC)

Table Shows reagents of SGPT in the kit

Reagent-1	**Concentration**
TRIS PH 7.5	100 mmol/l
L-Alanine	500 mmol/l
LDH (lactate dehydrogenase)	1200 U/l
Reagent-2	**Concentration**
2-Oxoglutarate	15 mmol/l
NADH	0.18 mmol/l
Good's buffer PH 9.6	0.7mmol/l
Pyridoxal -5-Phosphate	0.09 mmol/l

Assay Procedure

Mix 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube

a) To this, added 100 µlof serum.

b) Mixed well and took the reading immediately. Normal range: <41 u/l

B) ESTIMATION OF SGOT

Serum Glutamate Oxaloacetate Transaminase (SGOT)

Principle

Alanine aminotransferase (ALAT) and aspartate amino transferase (ASAT) are the most important group of enzymes of aminotransferase. These enzymes act as catalyst in conversion of a -keto acids in to amino acids by transfer of amino groups.

Increased levels of ALAT is found in the hepatobiliary disease condition where as increased ASAT levels occur in damaged conditions of heart and skeletal muscles as well as liver parenchyma. Parallel measurement of ALAT and ASAT is therefore applied to distinguish liver from heart or skeletal muscle damages

The ASAT/ALAT ratio is used from differential diagnosis of liver diseases

L-Aspartate + 2-Oxaloglutarate $\xleftrightarrow{\text{ASAT}}$ L-Glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{\text{MDH}}$ D- Malate + NAD^+

Method

Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)

Table shows Reagents of SGOT in the kit

Reagent-1	**Concentration**
TRIS Ph 7.8	80 mmol/l
L- Aspartate	240 mmol/l
MDH(malate dehydrogenase)	600 U/l
LDH(lactate dehydrogenase)	600 U/l
Reagent-2	**Concentration**
2-Oxaloglutarate	12 mmol
NADH	0.18 mmol
Good buffer pH 9.6	0.7 mmol/l
Pyridoxal-5-Phosphate	0.09 mmol/l

Assay procedure

a. Mixed 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube

b. To this, added 100 µl of serum

c. Mixed well and took the reading immediately

Normal range : <37u/l

C) ESTIMATION OF ALKALINE PHOSPHATASE

Principle

Alkaline phosphatase (ALP), hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver.

p-Nitro phenyl phosphate + water $\xleftarrow{ALP}$ Phosphate + *p*-Nitro phenol

Method

Kinetic photometric test, according to the international Federation of clinical chemistry and laboratory Medicine (IFCC).

Table Shows reagents of alkaline phosphatase in the kit

Reagent 1	**Concentration**
2-Amino-2-methyl-1-propanol pH 10.4	0.35 mol/l
Magnesium sulphate	2.0 mmol/l
Zinc sulphate	1.0 mmol/l
HEDTA	2.0 mmol/l
Reagent 2	**Concentration**
p-Nitrophenylphosphate	16.0 mmol/l

Test Procedure

Take 1000 µl of reagent-1 in a 5 ml test tube.

To this add 250pl of reagent-2 and mix well.

Add 20 µl of serum and mix well and take reading immediately and read absorbance at 405nm.

Normal range: 53-128 u/l.

D) ESTIMATION OF TOTAL PROTEIN

Principle

Protein forms a coloured complex with cupric ions in alkaline medium.

Table Shows reagents of total protein in the kit

Reagent-1	**Concentration**
Cupric sulphate	6 mmol/l
Potassium iodide	15 mmol/l
Reagent-2	**Concentration**
Protein (std)	6 g/100ml

Method: **Biuret method**

Procedure:

Preparation of test sample.

Take l ml of reagent-1 in a 5 ml test tube.

To this add 0.02 ml of serum.

Mix well and incubate at a room temperature for 15 min and read the test sample at 750 nm.Normal range: 3.2 to 4.2 g/dl.

LIPID PROFILES

A. ESTIMATION OF CHOLESTEROL

Cholesterol was estimated by the following method.

Reagent	**Concentration**
Ferric chloride-acetic acid reagent	0.05%
Cholesterol stock standard	1 mg/ml in acetic acid
Cholesterol working standard	40 a g in ferric chloride-acetic acid reagent
Concentrated sulfuric acid.	3.0 ml

Procedure

0.1 ml of extract was evaporated to dryness and 5.0 ml ferric chloride- acetic acid reagent was added, mixed and centrifuged. To the supernatant 3.0 ml of concentrated sulfuric acid was added and the absorbance was read after 20 min at 560 nm against a reagent blank. A Standard in the concentration range of 40-200 µg was treated similarly.

Values were expressed as mg/dL serum.

B. DETERMINATION OF TRIGLYCERIDES

Triglycerides were determined by the following method.

Triglycerides are extracted by isopropanol, which upon saponification with potassium hydroxide yield glycerol and soap. The glycerol liberated is treated with meta per iodate, which releases formaldehyde, formic acid and iodide. The formaldehyde released reacts with acetyl acetone and ammonia forming yellow coloured compound, the intensity of which is measured at 420nm.

Reagent	**Concentration**
Saponification reagent	0.6 ml
Sodium meta per iodate reagent	650 mg.
Acetyl acetone reagent	0.5 ml
Standard triolein solution	1 g of triolein was dissolved in 100 ml isopropanol

Reagents:

1. Isopropanol.
2. Activated aluminium oxide (Neutral).
3. Saponification reagent – 5 g of potassium hydroxide was dissolved in 60 ml of distilled water and 40 ml of isopropanol was added to it.
4. Sodium meta per iodate reagent - 77 g of anhydrous ammonium acetate was dissolved in about 700 ml of distilled water, 60 ml glacial acetic acid was added to it followed by 650 mg of sodium meta periodate. The mixture was diluted to 1 litre with distilled water.

5. Acetyl acetone reagent - 0.75 ml of acetyl acetone was dissolved in 60 ml of distilled water and 40 ml of isopropanol was added to it.

6. Standard triolein solution - 1 g of triolein was dissolved in 100 ml isopropanol. 1 ml of stock standard was diluted to 100 ml to prepare a working standard 100 µg of triolein/ml.

Procedure

An aliquot of serum/lipid extract was evaporated to dryness. 0.1 ml of ethanol was added followed by 4 ml of isopropanol. 0.4 g of alumina was added to all the tubes and shaken well for 15 min. Centrifuged and then 2 ml of the supernatant was transferred to labeled tubes. The tubes were placed in a water bath at 65µC for 15 min for saponification after adding 0.6 ml of the saponification reagent followed by 0.5 ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65°C for 1 h, the contents were cooled and absorbance was read at 420nm. A series of standards of concentrations 8-40 °g triolein were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 420nm.

The triglyceride content was expressed as mg/dL serum.

C. ESTIMATION OF HDL-CHOLESTEROL

Principle

The common classification of lipoproteins – HDL, LDL and VLDL comes mainly from ultra centrifugation of serum or plasma. HDL (specific gravity more than 1.063) can be separated by using polyionic substances along with bivalent metal ions. HDL is separated from other protein fractions by treating serum with

phosphotungstic acid and magnesium chloride. HDL remains in solution while all other lipoprotein fractions are precipitated. Centrifugation separates high density lipoproteins as a clear supernatant. Cholesterol content of which is estimated by enzymatic method as described earlier in estimation of total cholesterol.

Reagents

1. Enzyme reagent
2. Diluent buffer
3. Precipitating reagent PEG-6000
4. Standard – 200 mg/dL

The working reagent is prepared by dissolving enzyme reagent with 25ml of diluent buffer and kept for at least 10 min before use. The working reagent is stable for 4 weeks at 2-8^{0}C.

Procedure

0.5 ml of serum was taken into a glass tube and added 0.05 ml of precipitating reagent. Mixed well, and kept at room temperature for 10 min. The mixture was then centrifuged for 15 min at 4000 rpm. Separated the clear supernatant and immediately determined cholesterol content.

D. ESTIMATION OF LDL-CHOLESTEROL AND VLDL-CHOLESTEROL

The amounts of LDL-cholesterol and VLDL-cholesterol were calculated by using the Friedwald formula.

VLDL-Cholesterol = Triglycerides / 5

LDL-Cholesterol = Total-Cholesterol – (HDL + VLDL)

The levels of HDL, LDL and VLDL-cholesterol are expressed as mg/dL

Estimation of antioxidant parameters

On 11th day animals were sacrificed after blood withdrawal and abdomen was cut open and liver was dissected out.

The liver was perfused with 0.86% cold saline to completely remove all the red blood cells. Then it was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) Cut into small pieces, and the required quantity was weighed and homogenized using a homogenizer. The homogenate was centrifuged at 3000 rpm for 20 min to remove the cell debris. The supernatant was used for the estimation of Catalase, Superoxide dismutase, Glutathione peroxidase and Lipid peroxidase.

INVIVO ANTIOXIDANT ENZYME ESTIMATION

The homogenate was centrifuged and supernatant was used for the assay of marker enzymes namely,

Catalase (CAT)

Superoxide dismutase (SOD)

Lipid peroxidase (LPOX)

1. ESTIMATION OF CATALASE (CAT) ACTIVITY

Reagents:

Dichromate/acetic acid reagent (5% solution of potassium dichromate in acetic acid at 1:3 ratios)

0.01 M Phosphate buffer, pH 7.0

0.2 M Hydrogen peroxide

Procedure

Catalase(CAT) was estimated by the method of Sinha (1972).The reaction mixture (1.5ml vol) contained 1.0 ml of 0.01 M phosphate buffer (Ph7.0) 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).Then the absorbance was measured at 530 nm.

2. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Reagents

1. Adrenaline
2. Carbonate buffer (pH 10.2)
3. Mm EDTA

Procedure

The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar et al based on the oxidation of epinephrine adrenochrome transition by

enzyme. The post-mitochondrial suspension of mice liver (0.5ml) was diluted with distilled water (0.5).To this chilled ethanol (0.25ml) and chloroform (0.15ml) was added. The mixture was shaken for 1 min and centrifuged at 2000 × g for 10 min.The PMS (0.5ml) was added with PBS buffer (Ph 7.2; 1.5ml).The reaction initiated by the addition of epinephrine(0.4ml) and change in optical density (O.D.,min-l) was measured at 470 nm.SOD activity was expressed as U/l of tissue. Change in O.D (min-1) at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit.

3. ESTIMATION OF LIPID PEROXIDASE OF RAT LIVER

Reagents

1. Thiobarbituric acid 0.37%
2. 0.25N HC1
3. 15% TCA

Procedure

Lipid peroxidase in liver was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) using the method of Fraga et al. (1988). In brief, 0.l ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid -HC1 reagent (0.37% TBA, 0.25 M HC1 and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500 × g for 10 min at room temperature, the absorbance of clear supernatant was measured at 535 nm against a reference blank. Values were observed.

HISTOPATHOLOGICAL INVESTIGATIONS

A portion of liver processed for histopathological investigations.

Histopathological techniques

Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

Collection of materials

Thin pieces of 3 to 5 mm, thickness are collected from tissues showing gross morbid changes along with normal tissue.

Fixation

Keeping the tissue in fixative for 24-48 hours at room temperature

1. Serves to harden the tissues by coagulating the cell protein,
2. Prevents autolysis.
3. Preserves the structure of the tissue, and
4. Prevents shrinkage: The volume of the fixative added is 10 times the volume of the tissues. Common Fixatives: 10% Formalin

Haematoxylin and eosin method of staining

Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol, then wash in tap water. Stain with haematoxylin for 3-4 minutes and wash in tap water. Allow the sections in tap water 5-10 min and wash in tap water. Counter stain with 0.5% eosin until section appears light pink (15 to 30seconds), and

then wash in tap water. Blot and dehydrate in alcohol. Clear with xylol (15 to 30 seconds). Mount in Canada balsam or DPX Moutant. Keep slide dry and remove air bubbles.

STATISTICAL ANALYSIS

All the values of biochemical and antioxidant parameter estimations were expressed as mean ± standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test, using InStat v.2.02 and prism v.5 software (GraphPad Software Inc.).Differences between groups (p Value) were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The plant, *Girardenia zeylanica* Dcne belonging to family Urticaceae was selected for my project, on the basis of ethanobotanical information which reveals its uses against one of the most hazardous disease.

Girardenia zeylanica Dcne, is commonly called as Himalayan Giant Nettle; Nilgiri Nettle, is a perennial-rooted herb found abundantly in open forest land and river side of moist habitat in Himalayan parts of India like Uttarakhand, Himachal Pradesh and J&K and in high altitudes of Western Ghats and in Nepal.

Literature survey revealed that not much work has been done on this plant claiming maximum therapeutic uses. So we felt worthwhile to validate scientifically, the folk claim for its therapeutic activity. We have also taken its detailed Pharmacognostical and preliminary phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

1. PHARMACOGNOSTICAL STUDIES

ANALYTICAL PARAMETERS

The analytical parameters were investigated and reported as, total ash value (9.2 %w/w), water soluble ash value (3.4%w/w), loss on drying (5.11 %w/w), acid insoluble ash value (3.5 %w/w), sulphated ash value (5.1 %w/w), water soluble extractive value (3.7 %w/w), alcohol soluble extractive value (4.6 %w/w),. The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies.

The results were given in table.

1. Ash values

Table: 1. Data for ash values for powdered leaves of *Girardenia zeylanica*

S.No	PARAMETER ASH VALUES	%w/w
1.	Total Ash	9.2
2.	Water Soluble Ash	3.4
3.	Acid Insoluble Ash	3.5
4.	Sulphated Ash	5.1

2. Extractive values and loss on drying

Table: 2.Data for extractive values and loss on drying of powdered leaves of *Girardenia zeylanica*

Analytical parameter	Percentage (w/w)
Water soluble extractive	3.7 %
Alcohol soluble extractive	4.6 %
Loss on drying	5.11 %

PRELIMINARY PHYTOCHEMICAL STUDIES

The leaves of *Girardenia zeylanica* Dcne were subjected for hot continuous extraction using ethanol as solvent. The yield was found to be 5.27% w/w. the extracts obtained were subjected to various phytochemical tests, to identify the active constituents, which showed the presence of alkaloids, glycosides, flavonoids, and steroids, carbohydrate, fatty acids and proteins and phenolic compounds. The results were given in table: 3.

Table: 3. Results of Phytochemical analysis of powdered leaves of *Girardenia zeylanica*

PHYTOCONSTITUENTS	ETHANOL EXTRACT
Alkaloids	+
Saponins	-
Glycosides	+
Carbohydrates	+
Tannins	-
Flavanoids	+
Terpenoids	+
Steroids	+
Phenolic compounds	+
Proteins and amino acids	+
Fixed oils and fatty acids	+
Gums and mucilage	-

2. TOXICOLOGICAL STUDIES

Acute oral toxicity studies

The acute oral toxicity of *Girardenia zeylanica* Dcne extract was carried out as per OECD 423 – guidelines (Acute toxic class method).

No toxicity or death was observed for these given dose levels, in selected and treated animals. Hence, the acute toxicity studies revealed that LD_{50}> 2000mg/kg for the extract.

Hence, the biological dose was fixed at two dose levels, 100 and 200 mg/kg of body weight.

3. PHARMACOLOGICAL STUDIES

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

Assessment of serum biochemical parameters

Ethanol extract of *Girardenia zeylanica* Dcne possessed a good hepatoprotective activity on rats as shown in the table. At varying dose levels, (100 mg/kg and 200 mg/kg), this extract attenuated altered biochemical parameters produced by CCl_4 was dose dependent. This extract possessed significant hepatoprotective activity at both 100 and 200 mg/kg dose level.

Table-4. Effect of *Girardenia zeylanica* Dcne extract on serum parameters in CCl_4 induced hepatic damage in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TOTAL BILIRUBIN (mg/dl)	TOTAL PROTEIN (mg/dl)
GROUP I	97.26 ± 0.34	81.42 ± 0.13	141.9 ± 0.10	0.77 ± 0.003	10.04 ± 0.005
GROUP II	236.1 ± 0.20[a]	364.76 ± 1.27[a]	397.36 ± 0.12[a]	2.26 ± 0.006[a]	6.81 ± 0.009[a]
GROUP III	103.05 ± 0.09[a]	106.21 ± 0.13[a]	165.9 ± 0.10[a]	0.85 ± 0.005[a]	9.58 ± 0.003[a]
GROUP IV	206.13 ± 0.10[a]	210.20 ± 0.30[a]	215.7 ± 0.17[a]	1.71 ± 0.23[b]	7.53 ± 0.005[ns]
GROUP V	115.3 ± 0.28[a]	116.33 ± 0.62[a]	178.25 ± 0.11[a]	0.9 ± 0.005[a]	8.12 ± 0.52[b]

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at c=$p<0.05$, b=$p<0.01$, a=$p<0.001$. *Group II compared with group I and Groups III, IV & V were compared with group II.*

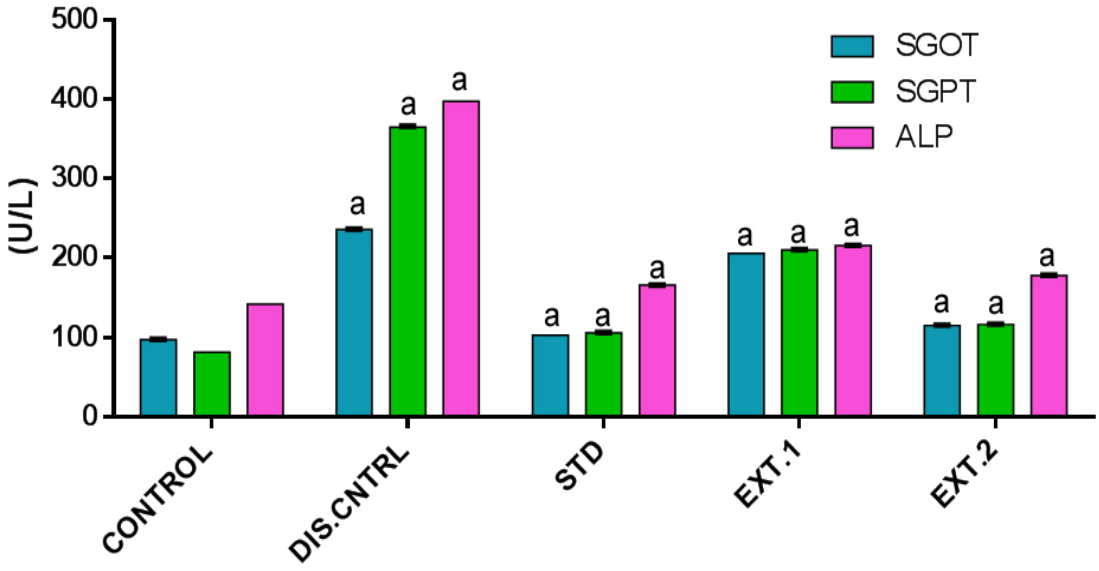


Figure-6

Effect of *Girardenia zeylanica* Dcne extract on serum parameters in CCl_4 induced hepatic damage in rats

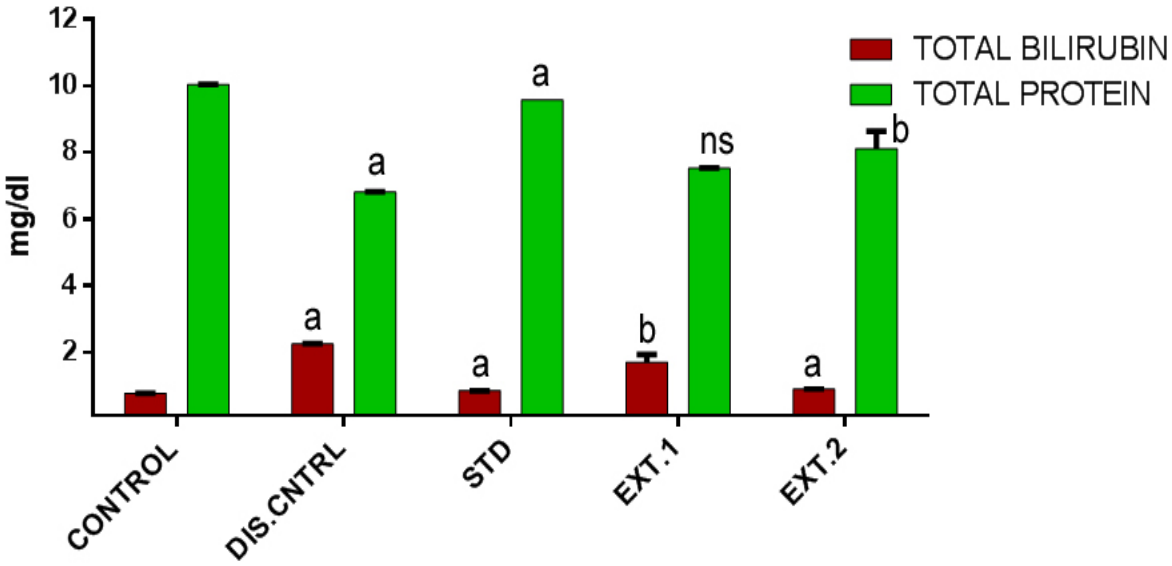


Figure-7.

Effect of *Girardenia zeylanica* Dcne extract on total protein and total bilirubin in CCl_4 induced hepatic damage in rats

Assessment of Lipid profiles

The serum lipid profile such as total cholesterol, triglycerides, LDL & VLDL were elevated, and this indicated deterioration in hepatic function due to the damage caused by CCl_4 administration.

Whereas treatment of *Girardenia zeylanica* Dcne extract significantly declined the effect of CCl_4 induced damage and it was evidenced by the decreased level of total cholesterol, triglycerides, LDL & VLDL and increased level of HDL in extract group.

Results were shown in table 5.

Table – 5. Effect of *Girardenia zeylanica* Dcne extract on liver lipid profiles in CCl_4 induced hepatic damage in rats

Treatment	CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
GROUP I	72.22 ± 0 .25	81.47 ± 0.12	40.49 ± 0.15	31.92 ± 0.15	13.01 ± 0.12
GROUP II	124.31 ± 0.34[a]	128.57 ± 0.62[a]	12.27 ± 0.09[a]	74.44 ± 0.11[a]	37.25 ± 0.13[a]
GROUP III	87.56 ± 0.62[a]	86.58 ± 0.15[a]	30.08 ± 0.06[a]	44.40 ± 0.06[a]	16.56 ± 0.12[a]
GROUP IV	121.01 ± 0.83[b]	125.24 ± 0.68[b]	25.27 ± 0.14[a]	47.59 ± 0.15[a]	20.38 ± 0.10[a]
GROUP V	102.29 ± 0.31[a]	104.24 ± 0.79[a]	28.79 ± 0.18[a]	39.28 ± 0.23[a]	13.51 ± 0.10[a]

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at c=*p<0.05,* b=*p<0.01,*a=*p<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.*

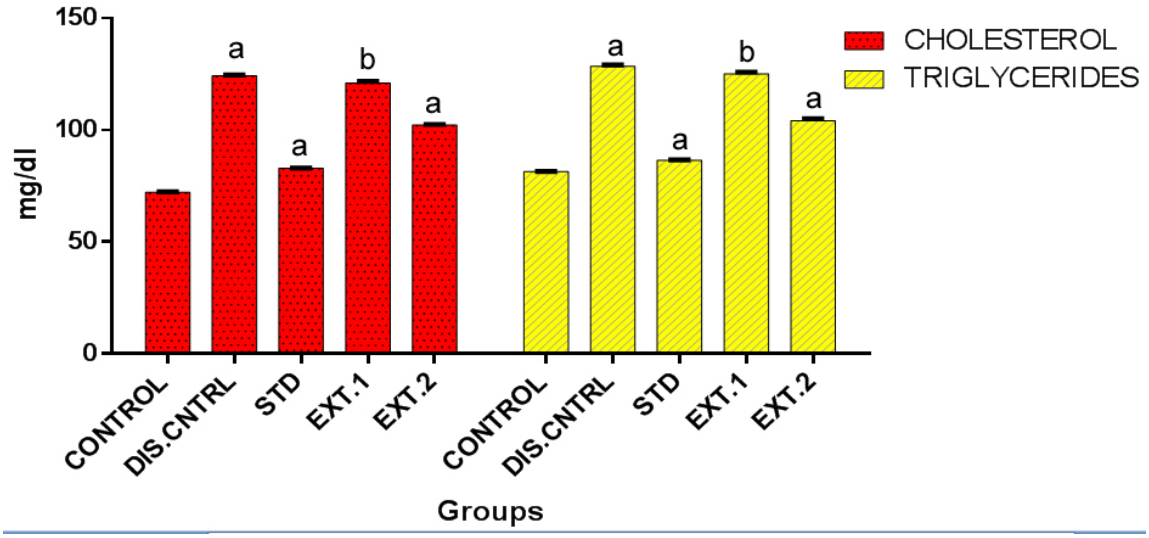


Figure-8.

Effect of *Girardenia zeylanica* Dcne extract on liver lipid profiles in CCl_4 induced hepatic damage in rats

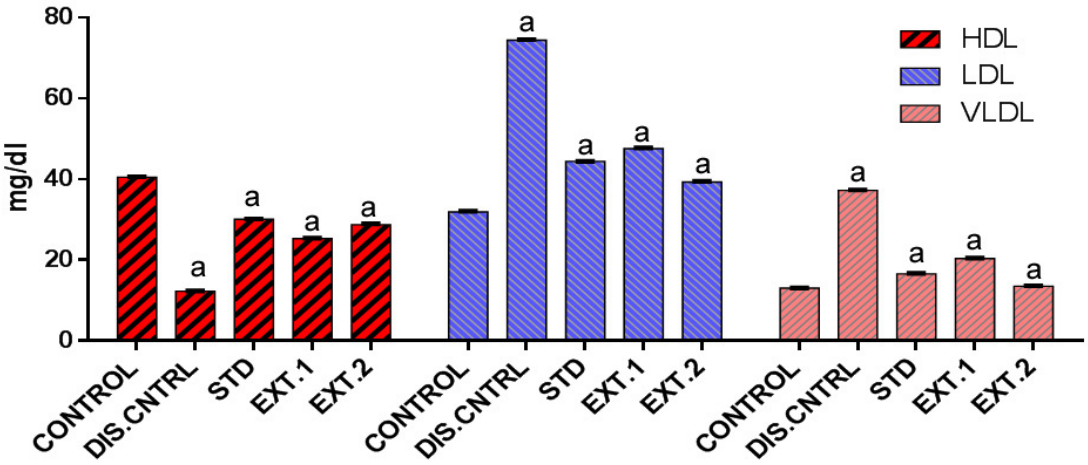


Figure-9

Effect of *Girardenia zeylanica* Dcne extract on HDL, LDL, VLDL in CCl_4 induced hepatic damage in rats

Assessment of antioxidant parameters

The effects of extract at two dose levels (100 and 200 mg/kg, p.o.) on liver antioxidant enzymes in CCl_4 -induced hepatic injury are shown in Table 2. Hepatic injury induced by CCl_4 caused significant increases in liver antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and lipid peroxidase. Administration of EEGZ at different dose levels shows significant dose-dependent decreases, when compared with diseased control animals. Results were shown in table 6.

Table – 6.Effect of *Girardenia zeylanica* Dcne extract on antioxidant parameters in CCl_4 induced hepatic damage in rats

Treatment	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)	Lipid peroxidase (nmol/mg of protein)
GROUP I	291.32±0.22	92.05±0.16	4.62 ±0.08
GROUP II	236.29±0.12[a]	75±0.07[a]	8.34±0.07[a]
GROUP III	286.94±0.04[a]	88.01±0.13[a]	5.72±0.08[a]
GROUP IV	239.17±0.12[b]	76.17±0.32[b]	7.73±0.21[b]
GROUP V	277.30±0.25[a]	85.34±0.24[a]	06.55±0.07[a]

Values are given as mean ± Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at c=p<0.05, b=p<0.01,a=p<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

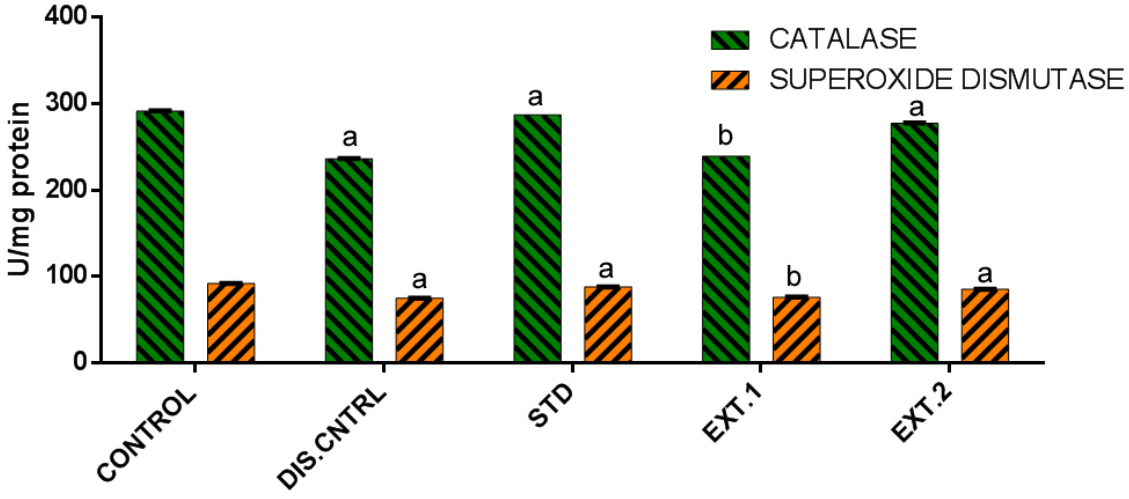


Figure-10

Effect of *Girardenia zeylanica* Dcne extract on Catalase and SOD in CCl_4 induced hepaticdamage in rats

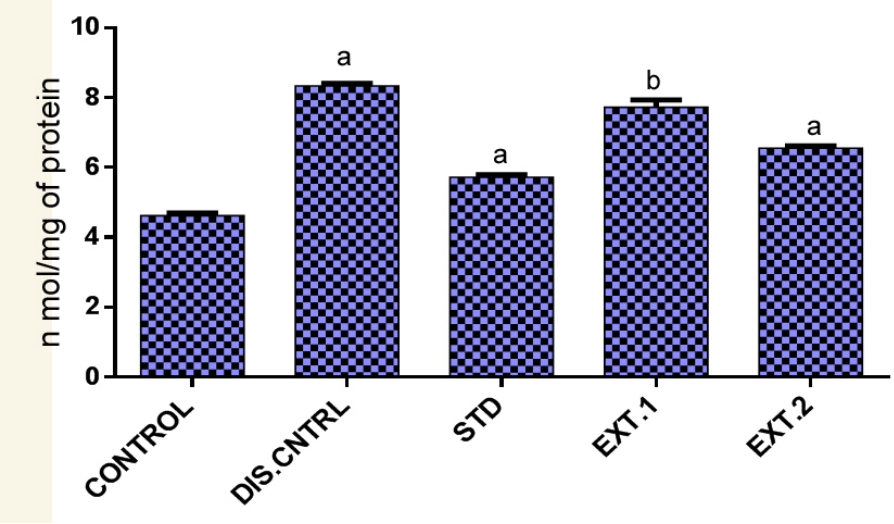
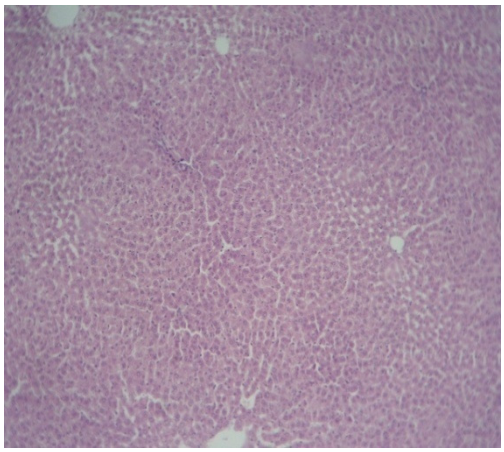


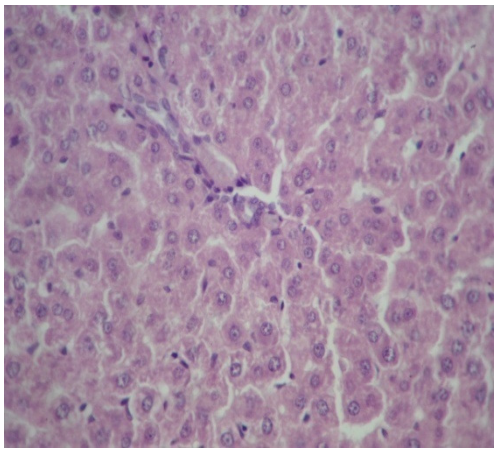
Figure-11

Effect of *Girardenia zeylanica* Dcne extract on LPOx in CCl_4 induced hepatic damage in rats.

Group I

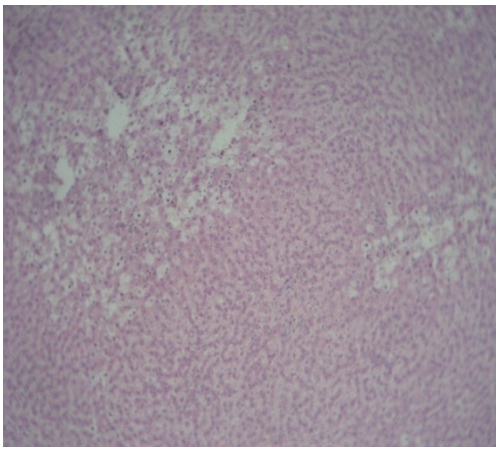


10x Liver with normal lobular architecture

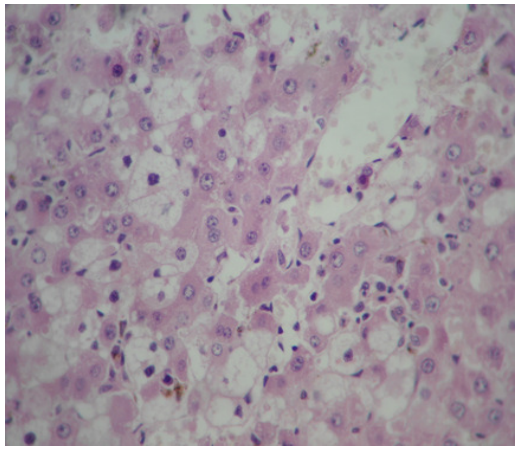


40x Normal portal tract and hepatocytes

Group II

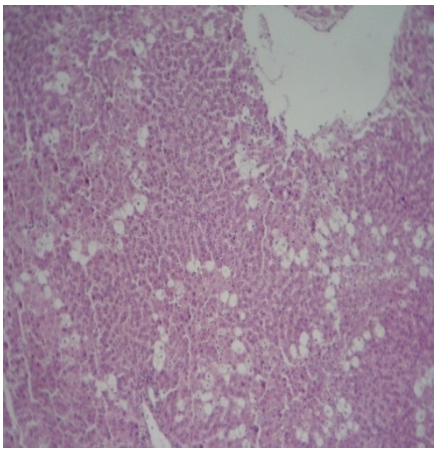


10x Liver showing parenchyma with fatty change(++++) and lobular inflammation

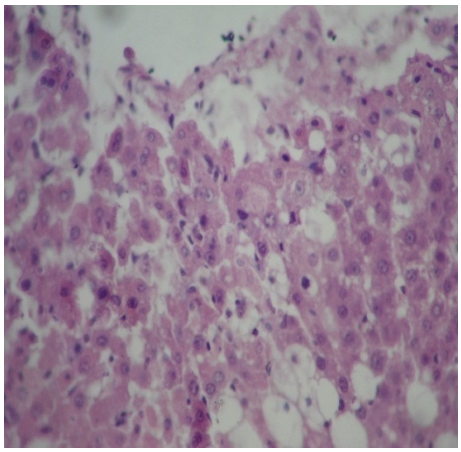


40x Perivenular region showing fatty change and lobular inflammation

Group III

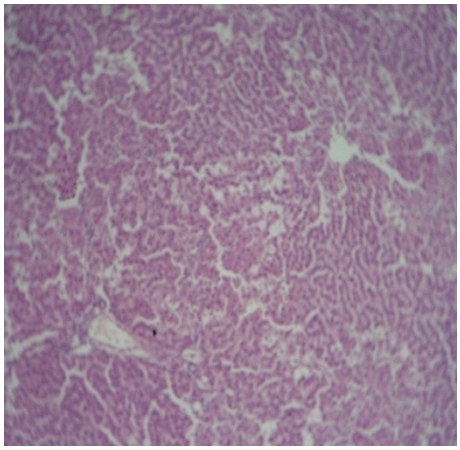


10x Liver showing parenchyma with fatty change(+)

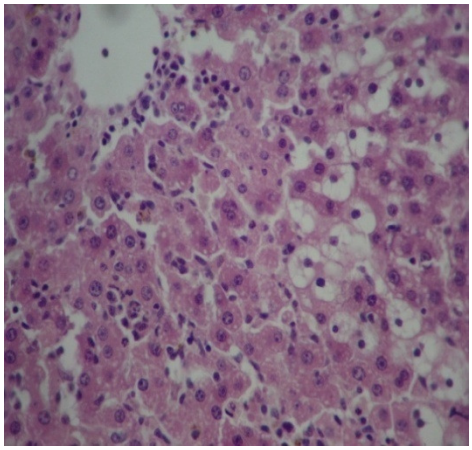


40x Perivenular region

Group IV

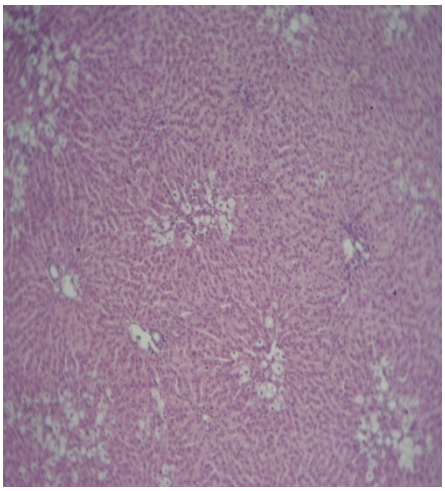


10x Liver showing parenchyma with fatty change (+++) and lobular inflammation

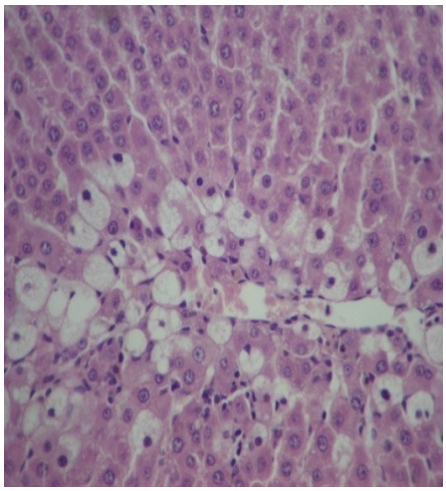


40x Perivenular region showing fatty change(+++) and inflammtion

Group V



10x Liver showing parenchyma with fatty change(+)



40x Perivenular region showing fatty change(+)

Figure-12.

HISTOPATHOLOGY OF LIVER OF RAT

Photomicrograph of liver shows a normal hepatic cellular arrangements in Group 1 (a) whereas, in group II (b) showing loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, crowding of central vein. In rats treated with silymarin (c), a normal hepatic architecture with moderate mild degree of necrosis. Group IV and group V (d & e) reduces the hepatic injury score of fatty degeneration and necrosis, clearly indicating the protection offered by EEGZ.

DESCRIPTION OF HISTOPATHOLOGICAL EXAMINATION

Group -I

Group I rat Liver tissue section shows normal lobular architecture of liver cells. Hepatocytes, hepatic sinusoids, portal tract, show normal size & Shape. The central vein and sinusoids are normal. There are no inflammation fatty change.

Group - II

Group II rat Liver tissue section shows extreme degeneration of hepatic architecture by necrosis, foci of hemorrhage, fatty changes, crowding of vein. The perivenular region shows extensive fatty changes with the areas of lobular inflammation with hepatocytes (fatty change++++, lobular inflammation ++).

Group -III

Group III Liver Tissue section shows mild degree of liver necrosis.Hepatocytes are compact. Hepatic sinusoids appear in normal. The Hepatocytes are well arranged like clusters. There is no inflammation seen. The portal tracts are normal (Fatty change +).

Group - IV

Group - IV Liver tissue section shows hepatocytes with fatty change. Hepatic sinusoids appear in normal. There are areas of lobular inflammation seen along with fatty changes in the perivenular region. There no fibrosis seen (fatty change+++, lobular inflammation +).

Group - V

Group - IV Liver tissue section shows that hepatocytes were regenerative and showed no visible changes and prominent nuclei, reduced score of necrosis and there is no inflammation. The portal tract are normal and there is no fibrosis seen. (Fatty change +, No lobular inflammation) Thus, confirming the safety of the extract.

DISCUSSION

Liver injury due to carbon tetrachloride in rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P 450 in endoplasmic reticulum and mitochondria with the formation of $CC1_3O^-$, a reactive oxidative free radical, which initiates lipid peroxidation and induces liver damage.

Damage induced in liver by carbon tetrachloride is accompanied by increase in the activity of some serum enzymes. The study of different enzyme activities such as SGOT, SGPT, ALP, TOTAL BILIRUBIN, TOTAL PROTEIN, CHOLESTEROL, TRIGLYCERIDES, HDL, LDL and VLDL have been found to be of great value in the assessment of clinical and experimental liver damage.

The liver is the largest organ in the vertebrate body, and is the major site of xenobiotic metabolism and excretion. Liver injury can be caused by toxic chemicals, drugs, and virus infiltration from ingestion or infection. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems. (Karan et al., 1999; Chaterrjee, 2000) Carbon tetrachloride (CCl4) has been widely used in animal models to investigate chemical toxin- induced liver damage. The most remarkable pathological characteristics of CCl4-induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl free radicals metabolized by the mixed function cytochrome P_{450} in the endoplasmic reticulum (Recknagel et al., 1989). Usually, the extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes (ALT, AST and ALP), thus leads to leakage of large quantities

of enzymes into the blood circulation. This was associated by massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver (Plaa and Charbonneau, 1989).

The aim of the present study was to investigate whether the extract from the plant *Girardenia zeylanica* possesses any preventive as well as curative role against CCl_4 induced hepatic damages. CCl_4 induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs and the extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase and transaminases in circulation. It is well documented that CCl_4 is biotransformed under the action of microsomal cytochrome-P450 of liver to reactive metabolites. These free radicals bind covalently to unsaturated lipid membrane, provoking a sharp increase of lipid peroxides followed by pathological changes such as elevated levels of serum marker enzymes like SGOT, SGPT and ALP, depletion of GSH, decreased protein synthesis, triglyceride accumulation, increased lipid peroxidation, destruction of ca2+ homeostasis and finally hepatocyte damage. This suggests that CCl_4 induces liver injury by sharing a common property of free radical mechanism.

Hepatocellular necrosis or membrane damage leads to very high levels of serum SGOT and SGPT released from liver to circulation. Among the two, SGPT is a better index of liver injury, since SGPT catalyses the conversion of alanine to pyruvate and glutamate, and released in a similar manner.

The activity of serum lipid profile such as total cholesterol, triglycerides, LDL & VLDL were elevated, and this indicated deterioration in hepatic function due to the damage caused by CCl_4 administration. Whereas treatment of *Girardenia zeylanica* extract significantly declined the effect of CCl_4 induced damage and it was evidenced

by the decreased level of total cholesterol, triglycerides, LDL & VLDL and increased level of HDL in *Girardenia zeylanica* treated group.

Necrosis or membrane damage releases the enzyme in to circulation and hence it can be measured in serum. A high level of SGOT indicates liver damage, such as that caused by viral hepatitis as well as cardiac infraction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore SGPT is more specific to the liver and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular damage and loss of functional integrity of cell membrane in liver. The ability of the ethanolic extract of leaves of *Girardenia zeylanica* prevent the increase in the activities of these enzymes is primary evidence indicative of hepatoprotective activity.

IN VIVO ANTIOXIDANT ENZYMES

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as Catalase, Glutathione peroxidase, Superoxide dismutase and Lipid peroxidase. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all tissues and the highest activity is found in red cells and liver. Catalase is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and OH.

Superoxide dismutase (SOD), a metallo protein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the ethanol extract *of Girardenia zeylanica* leaves, significantly increased the SOD activity in CCl_4 intoxicated rats there by diminished CCl_4 induced oxidative damage.

While SOD catalyzes the conversion of superoxide free radical to less toxic hydrogen peroxoide, GPx catalyzes the breakdown of hydrogen peroxide into water and oxygen and can also directly detoxify lipid peroxides generated by ROS(Castro andFreeman,2001). As SOD and GPx are easily inactivated by ROS or lipid peroxides, this may explain a decrease in activities of these two enzymes observed in liver tissue of CCl4 intoxicated mice in our study. However, our results demonstrated that SOD and GPx were appreciably elevated by EEGZ administration, suggesting that it can restore both enzymes and/or activate enzyme activities in CCl4-damaged liver tissue.

Lipid peroxidation has been postulated as being the destructive process in liver injury due to CCl_4 administration. Treatment with ethanol extract of *Girardenia zeylanica* leaves exhibited a significant inhibitory role against lipid peroxidation in rats and there by diminished CCl_4 induced hepatic membrane destruction and hepatic damage. The prevention of lipid peroxidation might, at least in part, be derived from the capability of *Girardenia zeylanica* leaves to scavange ROS.

The qualitative phytochemical analysis on the ethanolic extract of *Girardenia zeylanica* shows the presence of flavonoids. (C Pan *Et.al*, 2011).

The dominant constituents of *Girardenia zeylanica* are the flavonoids and triterpenoids. Flavonoid constituent of plant possess antioxidant and hepatoprotective properties (Wegner T and Fintelmann V, 1999).

SUMMARY AND CONCLUSION

The present study was undertaken to determine the hepatoprotective and antioxidant effect of ethanolic extract from leaf of *Girardenia zeylanica* Dcne.

The toxicity studies shows that the extract is not producing any harmful toxic effects within the dose range 2000 mg/kg.

The Pharmacognostical studies made on the powdered leaves of *Girardenia zeylanica* like ash values, extractive value, loss on drying gave valuable information. This will help for correct identification of the plant.

The preliminary phytochemical investigation showed the presence of, alkaloids, glycosides, flavonoids, and steroids, carbohydrate, fatty acids and proteins and phenolic compounds in ethanol extract.

Histopathological studies on isolated liver revealed that ethanolic extract of *Girardenia zeylanica* reversed the liver damage caused by CCl_4. The normal pattern of histology of liver was observed.

Phytochemical screening revealed the presence of flavonoids. Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect. Example: *Girardenia zeylanica contain* 2-di-C-glcosylflavones of vicenin and lucenin type, anthocyanin-cynidin-3-glucoside, luteolin-7-glycoside and mono-C-glcosyl flavones – vitexin and orientin.

Based on the results obtained from the present study, it can be concluded ethanolic extract of *Girardenia zeylanica* is found to be more potent and effective hepatoprotective and antioxidant activity.

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